FEDERAL SECURITY AGENCY

FOOD AND DRUG ADMINISTRATION

Microanalysis of Food and Drug Products

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Microanalysis of Food and Drug Products

I. INTRODUCTION

During the past year two manuals have been developed to assist analysts in the examination of samples of foods and drugs for the detection of violations of the Food, Drug, and Cosmetic Act. The first of these manuals, "Manual of Microanalytical Methods" consists of detailed instructions for the examination of foods and drugs suspected of being adulterated. This manual contains nearly all the microanalytical methods now in use by Administration analysts. Since the development of the manual some modifications in methods have been made and undoubtedly as time goes on revisions will be made and new and better methods included. The second of these manuals "Microanalytical Photomicrographs," consists of a group of photographs and photomicrographs depicting the various types of filth, decomposition, and adulterants that are encountered in the examination of foods and drugs. These authentic photographs have proven to be of considerable aid, particularly to the beginning analyst, in gaining knowledge in the field of microscopy.

In Part I of the Manual of Microanalytical Methods there was discussed the microscopy of foods and drugs. This discussion was given only in a general way since it was deemed inadvisable to attempt a full discussion of such a broad subject in a book on methods of analysis. The discussion given in the present book may be considered to be an extension of Part I of the book of methods. The data that are given are fundamental and should be of considerable help and interest to the analysts of the Administration concerned with the microscopy of foods and drugs. Since the microanalyst is entirely dependent on the inspector for the samples which he examines, and since the inspector will be interested in the fate of the samples which he collects, much of the material in the following discussion contains valuable information for the inspector. Because of the close relationship between filth analysis of foods and drugs and factory sanitation, a chapter dealing with sanitation has been included.

In developing the construction of this text the main object has been to correlate what the analyst observes microscopically in the food or drug product with authentic material. In doing this, there has been prepared for each station in the Administration a cabinet containing catalogued authentic material. The authentic material consists of var-

ious insect parts, rodent hairs, cat hairs, fibers, crystalline substances, etc., mounted on slides and Riker mounts of authentic material such as dried fruits, cocoa beans, nutmegs, etc. Where possible, camera lucida drawings have been made of the authentic material with a view to enabling the analyst to identify insect parts, and the like, in an adulterated product by comparison with the authentic material in the cabinet, and with this descriptive material.

The part of the text dealing with analysis of samples is arranged in the order in which the analyst normally carries out his examination, i.e., in the first section extraction is discussed, followed by the second section, "Identification of Filth." The actual detailed manipulations involved in filth extractions are given in the Manual of Microanalytical Methods, while in the present discussion an attempt is made to give some meaning to the procedures so that they can be carried out with intelligent understanding, rather than through some set of arbitrary rules. Moreover, as was recognized when the manual was first compiled, analysts of the Food and Drug Administration are continually meeting new problems in filth analyses, and it is not always possible to follow a rigid set of arbitrary rules. As many analysts have pointed out since the Manual was first compiled, on occasions it is essential for them to modify a method slightly. If the analyst has available a background of the theory involved in filth analysis, the needless repetition of fundamental studies will be reduced, if not eliminated.

There are occasional references to authentic material which has been supplied for official use to Food and Drug Administration field stations and similarly references are made to the Administration's Manual of Microanalytical Photomicrographs. None of this material is generally available to the public. However, the text has been so written that the above authentic material and Manual are not necessary for an understanding of the subject matter included. Where reference is made to specific microanalytical methods, these are given in the appendix.

The material included in the following text was prepared by the following microanalysts of the Microanalytical Division: W. V Eisenberg, K. L. Harris, W. G. Helsel, F. A. Hodges, G. L. Keenan, F. R. Smith, and J. D. Wildman. The chapter on sanitation was prepared in collaboration with Albert C. Hunter, Chief of the Division of Bacteriology.

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HENRY WELCH, *Chief,* MICROANALYTICAL DIVISION.

II. PLANT SANITATION

A. Object

Fundamentally, the noun "sanitation" and the adjective "sanitary" have a health connotation, and a sanitary condition, strictly speaking, is one which provides for freedom from contamination with injurious substances, particularly infectious micro-organisms. However, modern concepts of food and drug control undoubtedly go beyond the limited scope of this definition to encompass considerations of the maintenance of conditions which exclude the incorporation in foods and drugs of extraneous elements which are obnoxious and repulsive regardless of their lack of importance as agents of disease. Accordingly, an establishment operating in a manner to invite, or permit, contamination of its output with foreign matter properly classified as filth may be described as insanitary. The objectionable matter may be the excreta of man or animal or it may be in the form of maggots, worms, insects, and insect parts, some forms of trash and other material which disgustingly defiles the product. In many instances the contaminants are macroscopic and detection of avenues of their ingress into the product depends only on keen powers of observation and common sense. Other cases, involving microscopic filth, call for appraisal that invokes the inspector's knowledge and appreciation of invisible routes of distribution.

The likelihood that the factory output may be polluted or contaminated with filth is in proportion to the figurative distance between the source of filth and the product under preparation. An objective of the sanitary inspection is to measure this distance in terms of space, time, or vehicles of transmission. The distance between foul toilets and food may be great in space but significantly shortened by frequent and rapid movement of the carrier.

The most obvious is not always the most important. The problem is to differentiate between untidiness or disorderliness and conditions which contribute filth or the causative agents of disease. To quote Dr. Haven Emerson: "Let us deny ourselves the emotional indulgence of campaigns against nonessentials. Distinguish between the annoyance of noise, the unesthetic effect of untidiness, and the violence to health and safety in gluttony, self-drugging, and overcrowding. Let us get worked up over spitting, but leave litter abatement to housekeepers. Lend a sympathetic ear to those who believe their irritability results from other people's noise but raise particular hob if any privy is insanitary. Hold to the course of essential sanitation before sharing with those who fuss over outdoor dustiness and smells and robust racket." There may be a difference between plain dirtiness and insanitation. For instance, walls, ceilings, window glass, and door frames may be sadly in need of scrubbing but their unclean state is relatively unimportant as contrasted with the objectionable contribution made by flies, rats, and the behaviour of personnel. Lunch containers, pop bottles, discarded clothing, and loose storage of surplus equipment are signs of poor management, but not necessarily sources of filth. A foul odor is a clue to the existence of something wrong but of itself is not evidence; the source and cause must be found.

It is not necessary that the inspector be a bacteriologist, entomologist, parasitologist, or a sanitary engineer, but it is necessary that he make his inspection with some of the combined viewpoints of such professions. The burden of proof is to demonstrate routes of filth contamination and not to show that the establishment and its premises resemble a junk pile. Bacteriological concepts are helpful in appraising potentialities of infection with disease organisms or contamination with filth confirmed by bacteriological procedures.

B. Sources and Routes of Contamination

In enumerating the factors or elements to be evaluated in a sanitary inspection, it appears logical to discuss under one major heading the sources of filth and routes of contamination of primary importance and under another heading those features which are secondary but nevertheless important in filling out the picture. Matters of personal hygiene on the part of employees, prevalence of rats, mice and other vermin, incidence of flies, utilization of unclean equipment and polluted water or ice supplies, and the use of unfit raw materials comprise the first group.

The human element, although most difficult to control, cannot be subordinated to other factors in a sanitary appraisal. Man, en masse, is not clean. Civilization and education have taught decency but where education lags insanitary practices continue. In many large-scale operations, food handling is done by those who are not enlightened on the principles of personal hygiene. It is reasonable to demand that the food handler have clean hands, that he refrain from committing nuisances which spread filth, and that he acquire a consciousness that substances for human consumption are being prepared, to the end that his activities not defile those products. Medical certificates possessed by employees are no guaranty of good behavior and their ownership may be viewed with skepticism until knowledge has been obtained regarding the type of examination which led to their issuance and the length of time they have been held. In matter of dress, while there may be some transference of filth from clothing to the products handled, criticism of employee's attire should not unduly influence the conclusions reached regarding sanitation. Clean aprons, smocks, and caps have the advantage of creating in the workers' minds the attitude that they are clean, that dirt becomes quickly apparent on such garments, and that they should conduct themselves in accord with their dress. The inspector will undoubtedly give proper weight to exhibitionism, compliance with reasonable and decent standards, and displays of dirty attire that may have significance. Most sanitary codes contain prohibitions of human misconduct. However, such acts are transitory and difficult to detect by inspection. Efficient and continuing supervision is the remedy. The inspector can ascertain whether such supervision is provided for and whether the supervisor is fitted for his or her job.

From the beginnings of sanitary control efforts have sporadically been directed toward the elimination of rats and mice. Nevertheless, it has been estimated that in any given community the rat population equals that of the human inhabitants. Specific charges in the indictment of the rat as a destroyer and polluter of food might be multiplied indefinitely. Suffice it to say that the rat stands guilty as charged and

consequently merits careful attention in any worth-while sanitary inspection. Accumulation of fresh excreta, gnawings, rat runs, and damaged merchandise provide evidence of the prevalence of these invaders. Examination of the excreta will not only tell that infestation is occurring but will inform as to the extent and, from the size of the pellets, as to the existence of rat families. Attention to the existence of structural, incidental (furniture, fixtures, and equipment), and temporary (rubbish) harborages will frequently throw light on the possibilities of infestation. Hairs and excreta represent the types of filth sought as confirmatory evidence of depredations by rodents. If invasion by rats and mice cannot be completely controlled, it is obvious that raw materials and finished products should be stored to prevent access of the pests. Evidence of failure to provide adequate protection of the food or, better yet, evidence to show actual contact, furnishes demerits which weigh heavily in the sanitary appraisal.

Flies in appreciable numbers in a food establishment constitute twofold evidence of insanitation, since these insects have been shown to be carriers of filth and infection and their presence denotes the existence of putrescent or decaying matter in which they breed. Large numbers of flies indicate the presence of dunghills, unprotected latrines, or fermenting waste piles. The inspection should disclose the source and establish the evidence to show the relationship of flies to filth contamination of the food. In some instances confirmatory laboratory evidence can be obtained through the finding of insect parts in the finished product. The fly menace to the maintenance of sanitary conditions is a real one, but the evidence should be specific and reasonably detailed without recourse to such words as "teeming" or "swarming" unless conditions are so bad that no other terms apply. Adequate screening is, of course, the principal means of control. Fly poisons are not particularly efficient and may be a source of dangerous contamination. Fly ribbons and fly paper may be used to advantage but are unsightly. Chemical repellents are useful on waste piles but all recognized control measures are of no avail if conditions are generally insanitary. Dirty sinks, cesspools, open sewers, soiled containers, and decaying waste must be eliminated if control is to be truly effective.

Dirty utensils and equipment are a reflection of letdown in sanitary control. Some discretion is called for in determining to what extent the existence of pans and buckets with loose seams containing food residues, wooden equipment, soiled towels, and wash cloths may be cited as sources of filth. Certainly their use is to be decried and in connection with some products they assume an importance as vectors of bacteria which on laboratory examination serve to condemn the product. Generally, the disclosure of improperly cleaned equipment supports more direct evidence of filth. Whether or not utensils are dirty naturally depends on the facilities provided for cleaning them and a full evaluation of the sanitary picture will take note of the presence or absence of suitable water supplies and detergents. Disinfection of equipment usually means chlorination. This is to be recommended in food plants but not at the expense of thorough cleaning. On the other hand, failure to use chlorine is not of itself always a cause for condemnation. Too often hypochlorite solutions are used like cheap perfume on surfaces that might better be washed. Chlorine has a legitimate use in mantaining sanitary conditions but should be applied to clean surfaces and used in

effective concentration. The use of a steam hose is often resorted to for the treatment of heavy metal equipment. There is no reason to disapprove of the procedure but there is reason to belittle its efficacy as a disinfecting method, since the temperature reached by the spraying of flowing steam on cold metal is hardly sufficient to have much effect as a germ killer. Probably the best way to cope with the equipment disinfection problem is to maintain the equipment in clean condition by frequent and thorough washing, thus reducing to the minimum the numbers of organisms to be destroyed.

There should be little excuse for the use of polluted water in a food establishment. The bacteriological test for pollution is simple and will ordinarily be made by consulting laboratories for a reasonable fee or by local health agencies who may be in position to render such service. Except in rare instances, it may be assumed that a city water supply and ice manufactured from it are free from pollution. If water from a city supply is not available, it is pertinent to inquire regarding the sanitary quality of the water obtained from private wells or other sources. Procurement of water from open streams is not unknown. On general principles, since surface waters are practically always subject to intermittent pollution, the practice of using river or creek water constitutes an adverse item in the sanitary inspection report. While the use of polluted water may serve to add soluble filth to the food or to contribute a health hazard to those foods eaten uncooked, the difficulty, without the services of a nearby laboratory for the prompt examination of samples, lies in enforcement or compulsion of reforms. The burden then rests heavily upon the inspector who is called upon to exhibit his talents as a sanitary engineer. Attention should be given to possibilities of surface drainage, seepage from cesspools, pollution by animals and from sewage disposal; the inspector should ascertain the constancy and the degree of pollution. The gravity of the danger arising from a polluted water supply is something to be appraised in each case, taking into consideration the question of whether it is used in direct contact with the food or merely for washing down equipment. Proper control of the private water supply obviously resides in the location of a source of pure water, the maintenance of well-recognized safeguards against its contamination, or the institution of treatment such as chlorination.

From a broad viewpoint of plant sanitation, the fitness of the raw materials used is an item for consideration. Admittedly, evidence on the degree of infestation of fruits, vegetables, fish, and other commodities, serves to prove more directly contamination of the food previous to its entry into the plant. Nevertheless, the negligent and indiscriminate use of polluted or insect-and worm-infested raw material adds evidence to prove failure to observe proper sanitary measures. The impossibility of obtaining fish free from parasites or fruits and vegetables free from infestation may be recognized but a proper sanitary inspection will not overlook the effort, or lack of effort, exerted by the management to eliminate the filth in the process of preparing the finished product. In instances such as those where shellfish are obtained from polluted areas or vegetables are harvested from areas subject to sewage irrigation the inspection is automatically extended to include such evidence.

C. Supplementary Sanitary Features

The factors so far discussed are those likely to pollute or contaminate directly. However, the sanitary picture is not complete without information regarding those other features which contribute to the existence of insanitary conditions. For the most part, these comprise service facilities and structural features. Again to quote Dr. Emerson: "We cannot consider ourselves a civilized nation, nor imagine that we have attained even the safety of the Indian nomad with his shifting tepee, until each settlement, shack, house or place of human habitation and use is so equipped that family and friends, guests, laborers, and travelers can meet the calls of nature without soiling the spot of land they occupy with infecting and infesting organisms."

This quotation bespeaks the importance of the toilet in any sanitary set-up. It may not be necessary to specify the number of toilets which should be provided but those installed should be readily accessible. Inaccessibility leads to the committing of nuisances. To be sanitary it is not required that toilets be flush type connected with a sewage disposal system. Privy vault toilets are acceptable if properly constructed and used so that filth is not transferred to the product prepared in the establishment. It is hardly necessary to emphasize the need for cleanliness of the toilets in view of their importance as potential sources of pollution. They should be well screened and equipped with tight walls and floors and with self-closing, tight-fitting doors.

Washing facilities rate with toilets as a prime essential for the personal hygiene of workers. They should be adequate in number for the needs of the employees and should be installed in conspicuous locations so that the employee does not have to hunt for a place to wash his hands. Running water, heated if possible, is essential. The barrel or tub into which all employees immerse their hands serves no good purpose. Soap in some form should be provided. The matter of towels gives rise to debate. It is better to have none than to present for use a dirty community towel long since viewed with disapproval by public health officials. Paper towels, although expensive and apt to be wasted, are in keeping with modern sanitation. To the extent that unclean toilets and lack of washing facilities provide definite possibilities for the pollution of the output of the plant, they constitute important features of the sanitary inspection.

No sanitary inspection is complete that omits attention to the plant surroundings. In actual practice the inspection starts with a survey of the adjacent premises. Manure piles, accumulations of plant waste and pools of stagnant water, all of which furnish breeding spots for insects, sewer outfalls in position to pollute the water supply, dilapidated wharves, vacant and abandoned shacks and litter and rubbish heaps, all affording harborage for rats, are elements which singly or in combination will lower the level of sanitation in the establishment. Whereas it is hardly possible to state categoricaly that nearby dwellings are a menace to plant sanitation the nature of those dwellings and their capacity to produce dogs, cats, and stray children to roam at will through the plant are matters for appraisal. If the inspection discloses that dwellings are a menace to sanitation the report should show clearly in what way the pollution occurs. In areas where oysters are grown, or plant products such as water cress are produced, pollution attributable

to drainage from barnyards, toilets, pig pens, and the surrounding terrain, is to be considered. In evaluating the fitness of an area for food production, conditions should be taken at their worst and not their best. Possibly it is superfluous to state that evidence regarding surrounding premises is primarily background for the picture containing more vivid elements.

To the extent that plant structure has a bearing on the likelihood of rat infestation or interferes with proper cleaning this feature becomes important. However, it is a basic precept in food sanitation that clean products can be produced with clean methods in an old and, at first impression, unfit, building whereas beautiful buildings are totally without effect when insanitary procedures are followed. Perhaps the greatest concern is with the arrangement of equipment and installations to permit proper cleaning and with screening and rat proofing. Generally it may be borne in mind that the type of buildings and the materials of which it is constructed are of secondary importance.

A discussion of plant "cleanliness" reverts to previous discussions of differentiation between unclean conditions and those which render a product filthy or dangerous. There is the possibility that dirty floors, walls, ceilings, benches, and chairs may contribute filth to products prepared in the plant but the route of contamination is indirect. A keen sense of values is required of the inspector. What might constitute danger of filth in one type of plant would be of no great significance in another. Failures to provide water under pressure, steam, brooms, and mops and, when they are provided, to use such facilities frequently and with intelligence, build up a story depicting an improperly maintained establishment. The importance of such failures in any given case will depend upon circumstances and the information obtained to show their connection with contamination.

Adequate light and sanitation are requirements of all sanitary codes. Just how much light and fresh air may be deemed adequate for the comfort and well-being of employees is a question for specialists in that field. From the sanitary viewpoint the amount of light provided should be that needed to sort out unfit materials and see the way to do a clean job. Apart from the psychological and human physiological aspects, sufficient exchange of air should be maintained to dry out damp areas and thus hold in check moldiness and the development of insect pests. Actually lack of light and fresh air do not directly establish insanitary conditions. As items in a report they are worthy of note for the effect that failures in this connection have on the operations of the plant.

Waste disposal is definitely an important factor in sanitation. Improper disposal may merely create an unsightly and odoriferous nuisance. It is more likely to create a breeding place for rats and vermin. Obviously in the operation of some food establishments, an oyster shucking house, for instance, waste will unavoidably accumulate. The waste disposal problem must be viewed with a practical eye and appraisal made by giving due weight to the probabilities and improbabilities of pollution or contamination from that source. Certainly when the refuse pile putrifies or ferments, breeds flies, or serves as a harborage and food reservoir for rats it must be given a high rating as an insanitary factor.

In considering sanitation of growing or producing areas the discussion moves from sanitary "inspections" to sanitary "surveys." The contributing sources become more widespread and may vary according to weather conditions. Such surveys ordinarily cannot be made by a single inspection. They require sufficient time to obtain data under all conditions and may call for substantiating laboratory findings. Ordinarily a sanitary survey of a water shed, a shellfish-producing area, or a farm or series of farms producing dairy products or vegetables is a job of considerable magnitude and not likely to be undertaken on a routine regulatory basis.

D. Bacteriological Aspects

A sanitary inspection is usually accompanied or followed by the collection of samples. Where the extraneous matter to be sought by laboratory methods is macroscopic the good judgment of the inspector, based upon his direct observations, will dictate a proper collection of factory samples. To obviate misunderstandings relative to the collection of samples for bacteriological examination a few comments are presented regarding the usefulness and limitations of coliform bacteria as indices of pollution and possible danger to health. The coliform group is probably much overworked as an index of pollution, but when viewed in its proper perspective has great value in pointing the way to possible sources of contamination. This group of bacteria, comprising known species of fecal origin and species which may or may not have their natural habitat in the intestines of warm-blooded animals, is ubiquitous and its distribution in nature cannot be restrained to that degree which permits dogmatic conclusions that the presence of any number of the group is sure-fire evidence of pollution. In order to interpret the findings of *Escherichia coli*, the member of the group of known fecal origin on which regulatory actions are sometimes taken, a background of correlative data is needed. With knowledge that crabmeat and shelled nuts are free from *Esch. coli* as they start through the processing plant this organism serves satisfactorily as evidence of pollution when such evidence is supported by inspectional data to explain the method of its access to the food. In the control of oysters and water it is also useful with certain limitations. Examination of a food product for the presence of *Esch. coli* is of value only when the history of the product is known or can be ascertained. Similarly, the examination of specimens from a plant is of value only when there is evidence, or when evidence can be obtained, to indicate pollution with the excreta of warm-blooded animals, including man. It must be borne in mind that the test for *Esch. coli* is a test for fecal pollution.

Where there are considerations of possible danger to health in the pollution of a food product thought is directed toward the likelihood of finding the causative agents of the so-called filth diseases such as typhoid and paratyphoid fever, bacillary dysentery, amebic dysentery and gastro-enteritis (food poisoning). The detection of such pathogens is difficult, indeed, rarely possible and *Esch. coli*, not ordinarily regarded as a pathogenic organism, must be relied upon as an index of danger. It is then reasonable to argue the well-established principle

that substantial numbers of *Esch. coli* with adequate inspectional evidence to point out the probable source indicate a potential danger in the consumption of the food. The points to which this discussion is directed are that inspectional evidence should throw light on the avenues of pollution, that correlative studies should lay the basis for proper interpretation and that sampling should be carried out with full appreciation of the nature and purpose of the bacteriological examination.

Following the acquisition of the data during the sanitary inspection a report must be submitted to place on record a complete picture of the elements which characterize an establishment as insanitary. This means coming to the essential points promptly and by careful selection of language with the avoidance of lurid and extravagant terms depicting those conditions which justify the conclusions. For some parts of the report forms are useful but since no set form can be prepared to cover individual plants a true word picture presented in narrative style is usually necessary. For the inspector's own records and for the use of those who are chosen to support his conclusions it is essential that the exposition of data shall be clear and concise and should place emphasis on the significant elements, relegating to the appendix minor matters which fill in the background of the picture.

This discussion of plant sanitation has omitted some matters that are niceties of construction and operation but not necessarily matters of sanitation bearing on filth. Drinking fountains, the use of white paint, cement construction, and the maintenance of rest and recreational facilities for employees fall in this category. The purpose of the chapter has been to discuss principles of sanitation and routes and sources of contamination with suggestions as to what may be deemed important and what may be held to be relatively unimportant with full recognition that no Manual or text can take the place of actual experience.

III. RODENT CONTAMINATION

The rodents constitute the largest order of mammals and of this order the family Muridae (muskrats, lemmings, mice, and rats) represents about one-fourth of the American mammals. Where precautions are not taken, rats and mice breed prolifically and frequent any place where food can be secured. They eat practically any of the foods man himself eats and may be found in granaries, dried food plants, in canning and packing establishments, and in the field, eating the food and rendering it filthy by their chewing, voiding of solid and liquid excrement, and dropping of hairs and other filth in and on the food. Rats and mice are among the most filthy of animals and in addition to the repulsiveness caused by their mere presence they may carry disease-producing micro-organisms. However, sanitation itself has been treated as a separate subject and it is important here to treat only the diagnosis of rodent infestation in the plant and comminuted in the food.

There seems to be little difficulty in distinguishing RODENT EXCRETA PELLETS from the nondescript filth that may be found either around a food-packing establishment or in the food itself. However, care should be taken that COCKROACH EXCRETA, which is somewhat the same size as small mouse pellets, is not mistaken for that of mice and rats. Rodent excreta, of course, may be identified by its size and shape (see Figure 1), by the fact that it has a mucous-like coating, and by the EMBEDDED RODENT HAIRS. In infested plants rodent excreta is usually found along the walls, behind sacks or in any storage space that is not regularly cleaned. It should be borne in mind that the rodents need both food and water and their pellets may be found adjacent thereto. At any rate, suspected rodent excreta pellets always should be checked for the presence of rodent hairs.

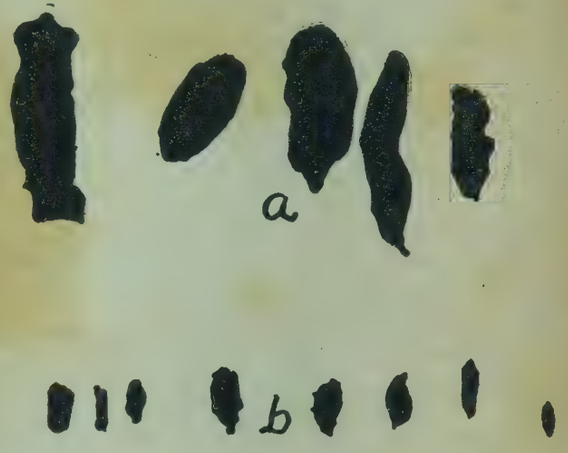

FIGURE 1.—Shadow profiles. *A*, Rat excreta; *B*, Mouse excreta.

In addition to feces, urine may be voided by rats, mice, and cats in food-processing establishments. The urine stains appear as somewhat darkened soiled areas on or about the food, or on the sacks, boxes or other containers. Urine fluoresces under ultraviolet light. When there is any doubt as to the nature of suspected stains, portions of the stained material can be removed to the laboratory for a confirmatory examination for the presence of urea.

BIRD EXCRETA also may occur where food is being stored or processed. However it is readily distinguished by its white, chalky matrix. In certain more or less hard substances such as cheese, dried fruits, nuts, grains and spices the RODENT TEETH MARKS (see Figure 2) sometimes may be detected. These teeth marks are usually short parallel scratches or indentations spaced by high sharp ridges and are made by the rodent gnawing with the two sharp incisors in the front of the mouth.

In the case of a large block of food such as cheese, small holes or pits may be eaten out, while in small particles, the corners or edges are usually nibbled. Shelled corn often is damaged by the animal eating out the germ and leaving the remainder of the grain intact. In ear corn the ear may be "blazed" by having the tops eaten off the grains over a considerable area. When cloth bags are gnawed the edges of the holes have a PECULIAR, SHREDDED, FRAYED, OR RAGGED APPEARANCE caused by the teeth of the rodent tearing and pulling at the threads. Wooden boxes or bins, partitions, walls, floors and other woodwork often show evidences of rodent gnawing. As a rule these gnawed holes

FIGURE 2.—Cheese showing rodent-gnawed areas and tooth marks.

are located under a door or in a corner or where a small crack or hole previously existed. The edges of the hole usually are beveled off and often the floor area leading to the hole will be stained. These stains are from the oily secretions from the feet of the animals. Similarly the top and the side of the hole will have a greasy, soiled appearance where the fur has rubbed. Cats may be present around a plant and have been known to deposit their excreta in piles of grain in the same manner as they would normally deposit it in dirt. Both dogs and cats may leave their hairs in the food product. Chickens and other poultry and birds also may be found where food is being handled and unless precautions are taken to keep them out they may deposit excreta in or near food or their feathers may fall into the food and may be found in a subsequent examination of the product. Fortunately, RODENT EXCRETA PELLETS have certain definite characteristics which are maintained even when the pellets are FRAGMENTED. The characteristics

depend somewhat upon the food the animal has eaten. For example, when grain is being consumed particles of the bran or hull tissues are found in the excreta along with sand or dirt and a few insect fragments. The rat excreta is coated by a mucous-like secretion which sometimes may be present even when the pellets are in a comminuted condition and is seen as a grayish-white layer when the fragment is moistened. For an exact determination, however, we must IDENTIFY the EXCRETA FRAGMENTS by the presence of RODENT HAIRS. (See slide I-2). The hairs of most animals such as man (Figure 3), dogs, and cows,

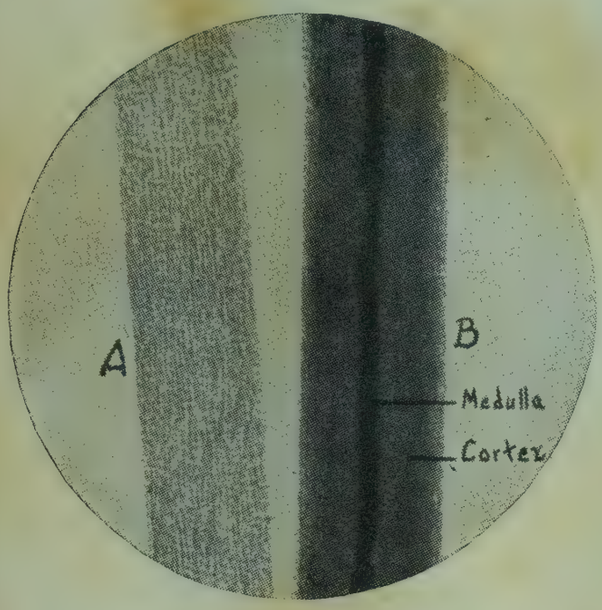

FIGURE 3.—Human hair. *A*, Blond, with little pigment in cortex, no medulla apparent. *B*, Brunette, with heavily pigmented cortex and medulla present.

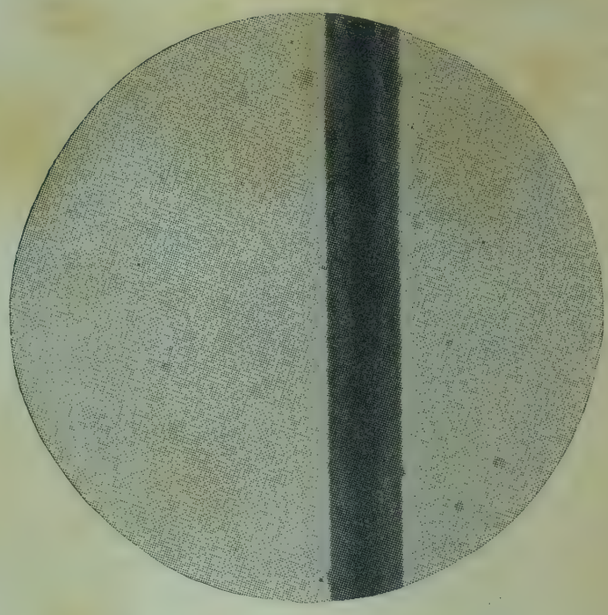

FIGURE 4.—Red cow hair. Medulla continuous, pigment in both cortex and medulla.

(Figure 4) have a continuous medulla and are readily distinguished from rodent hairs, and the only difficulty lies in the fact that there is a marked similarity between certain cat and rodent hairs. To explain better the characteristics of rodent hairs it is necessary to explain certain features common to hairs in general.

ANIMAL HAIRS may be divided into four general classes: (1) Guard hairs, or over-hairs, which are coarse, heavy hairs that in the living animal act as protection for the softer hairs; (2) soft, under, or fur, hairs which are soft fine hairs used to keep the animal warm; (3) intermediate or curly over hairs which have the characteristics of both guard and fur hairs alternating along their length, and (4) tactile hairs such as the vibrissae of animals. Tactile hairs are rarely found in food products and will not be treated further.

All animal hairs, whether they are fur or guard hairs, exhibit the following structures (Figures 5, 6, and 7):

(1) They are covered with a cuticle composed of overlapping keratinous scales of variable shapes. The shape of the scales sometimes is of value in identifying some types of hairs, but in cat and rodent hairs this feature is of limited importance; (2) inside the cuticle the hair contains a cortex. This area may or may not contain some pigment granules and in some hairs it may appear to be evenly stained with color because of the presence of very fine pigmentation. The size and

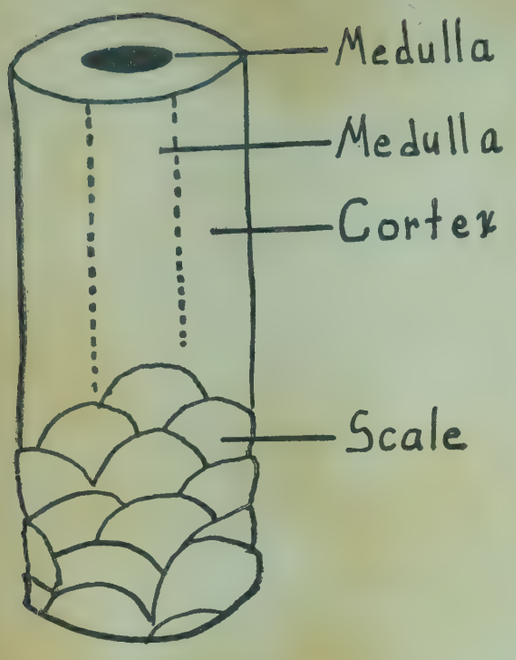

FIGURE 5.—Diagram of hair showing the overlapping scales, cortex, and central medulla.

density of the pigment granules, as well as the size of the cortex and relative sizes of the cortex to the underlying medulla, sometimes are of diagnostic value; (3) inside the cortex the hair contains a medullary shaft. It is composed of various loosely arranged cells, interspersed with air spaces. In some hairs the pigmented areas and the air spaces give a characteristic microscopic pattern to the medulla and in other hairs the loosely arranged cells are not noticeable and the cortex appears to be a tube with a hollow medulla.

The color effect of hair is based on two different factors, air spaces and pigment granules. The air spaces, when examined microscopically with transmitted light, appear to be black because the light is reflected within them and does not pass on through. If the air spaces are filled with balsam or some other substance, the hair appears almost completely clear; if some true pigment is present, the pattern of distribution of the pigment may be distinctly seen. When pigment is present in the cortex it is present as true pigment granules, but in the medulla it may be present as true pigment or confused with air spaces. In the examination of a hair or hair fragment to determine whether it is cat or rodent, the first character to note is the medulla. If the medulla is multiple-rowed, discontinuous, it is the guard hair of a rodent (Slide I–3, Figure 8); if it is smooth or erose (continuous), it is the

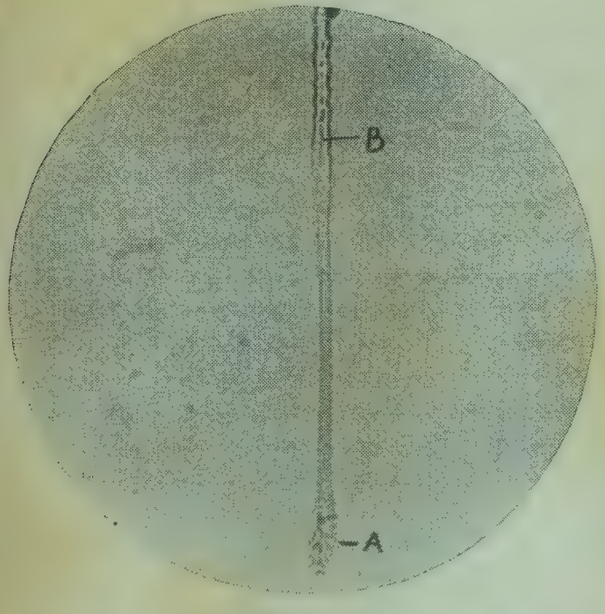

FIGURE 6.—Root of a rodent hair. *A*, Vascular papilla; *B*, Beginning of medulla. Hair from A to B is within the hair follicle. Surface of skin is at B. The hair grows from the papilla.

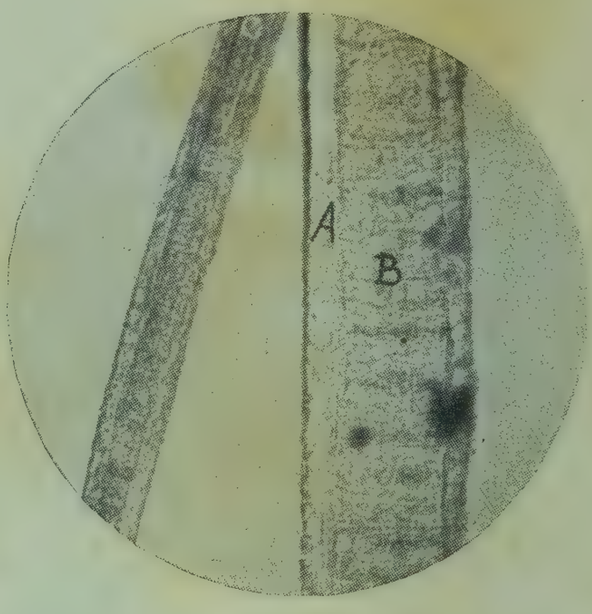

FIGURE 7.—Calf hairs. The cortex and medulla show in optical section. *A*, Cortex; *B*, Medulla.

guard hair of a cat (Slide I–8). It is only when the hair has a single-rowed, discontinuous medulla (Slides I–4 and I–5, Figure 9) that dif-

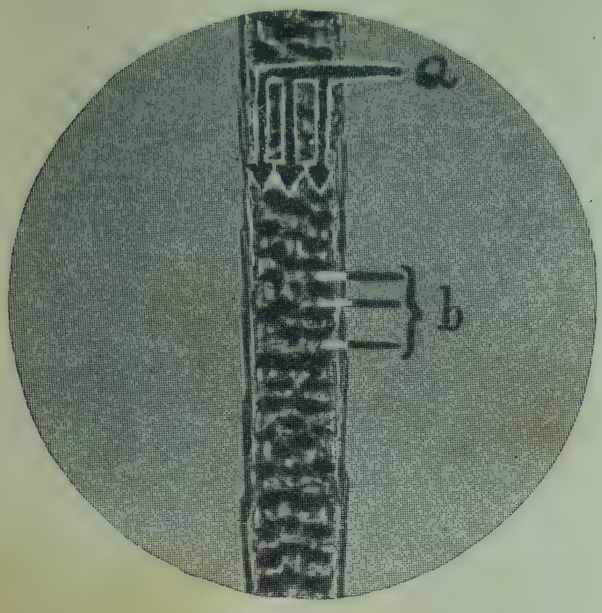

FIGURE 8.—Rodent guard hair showing characteristic multiple-rowed discontinuous medulla. *A*, Arrows indicate multiple rows; *B*, Discontinuous pigment effect.

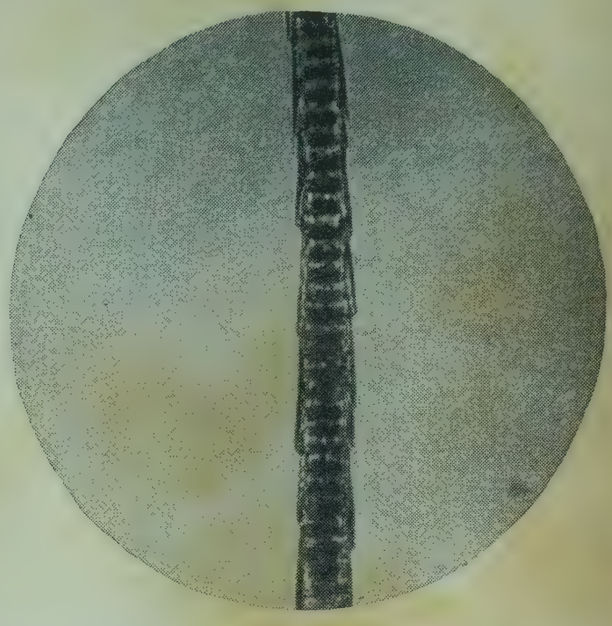

FIGURE 9.—Hair showing single-rowed discontinuous effect.

ficulty in differentiation is encountered. This type of medulla occurs in the fur hairs of both rodents and cats. Fortunately, along the length of the hairs of these animals there are a number of constrictions or internodes (Slide I-4, Figure 10).

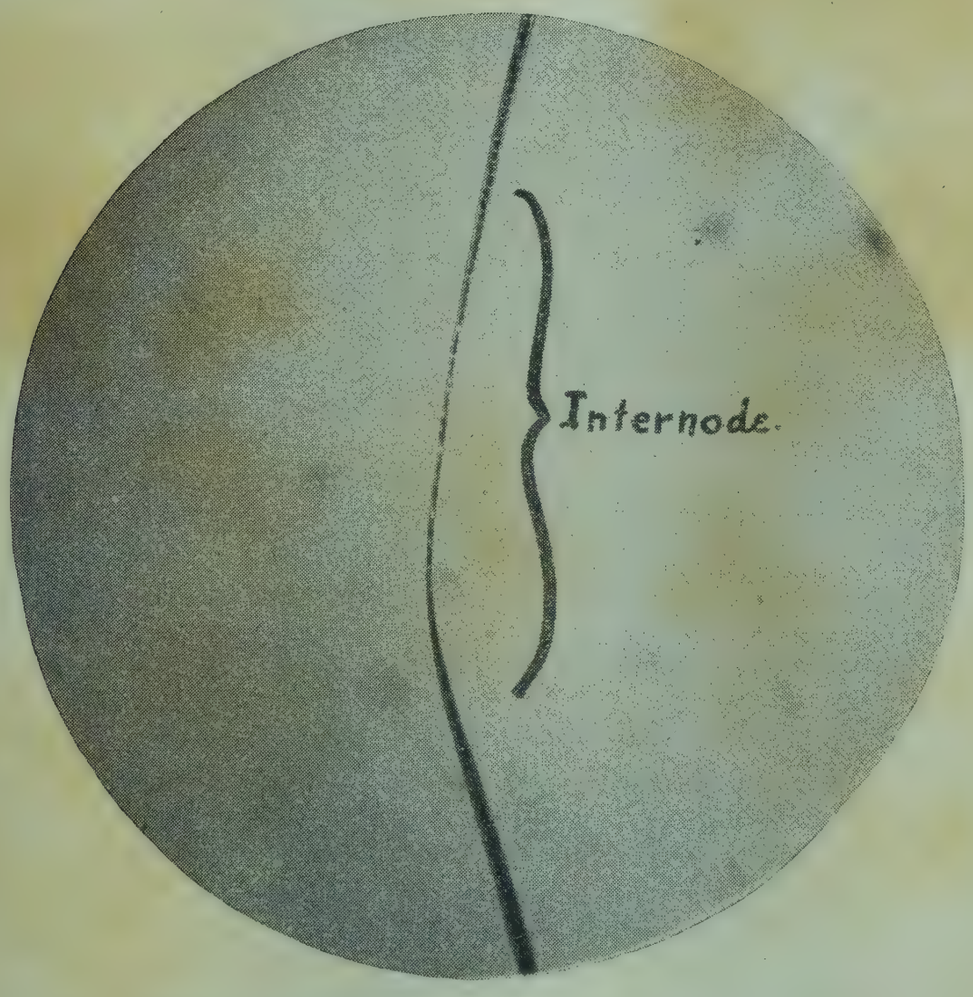

FIGURE 10.—Prominent internode of rodent hair.

THE INTERNODES OF OUR COMMON HOUSE MOUSE AND BROWN RAT USUALLY ARE VERY PROMINENT, WHILE THOSE OF THE CAT ARE MUCH LESS PROMINENT. Unfortunately, the internodes of some rats are not overly pronounced. For example, approximately 10 percent of the last internodes toward the proximal end of the hairs of brown rats are as indistinct as the more distinct ones of the cat. The internodes of the black rat similarly are about as indistinct as those of the cat and, therefore, are frequently difficult to differentiate. However, the black rat is common only in a few coastal localities in this country and this factor is of relatively small importance. In any case it is apparent that if a prominent internode is present, the hair is that of a rodent, but if the hair fragment is short and the internode is either indistinct or lacking, further study may be necessary.

In general CAT HAIRS ARE LARGER in diameter than either rat or mouse hairs, but when individual measurements are made it is found that considerable overlapping occurs.

The CORTEX OF CAT HAIRS IS THICKER GENERALLY than that of rodent hairs and the relative size of the cortex to the medulla often shows the cortex of cat hairs to be relatively thick while that of rodents often is relatively thin. Again actual measurements have shown that some overlapping of the character occurs, and it should be used only in conjunction with other features.

Cortex pigment is of more common occurrence in the hairs of cats than it is in those of rodents. Usually, when large numbers of cortex pigment granules are present, the hair is that of a cat rather than of a rodent. When pigment is present in the cortex of a rodent hair there will invariably be some in the medulla and WHEN PIGMENT IS PRESENT IN THE CORTEX ONLY, THE HAIR IS DEFINITELY THAT OF A CAT. Most cat fur hairs have more densely concentrated pigment granules than most rat or mouse hairs. At 200–400 x this pigment is visible as a blotched darkening of the cortex. At 800–1000 x (oil immersion objective) the pigmentation resolves itself into distinct and separate granules. Hairs swollen in NaOH solution or chlorine (hypochlorite) solution and then examined using the oil immersion objective show the granules even more plainly. In cat hairs (see Figure 11) they are arranged in short broken parallel rows while in rat and mouse hairs the rows are usually widely separate and less obviously parallel. Granules are ovate-elongate to round in both types of hair, although there may be more elongate granules in cat hairs.

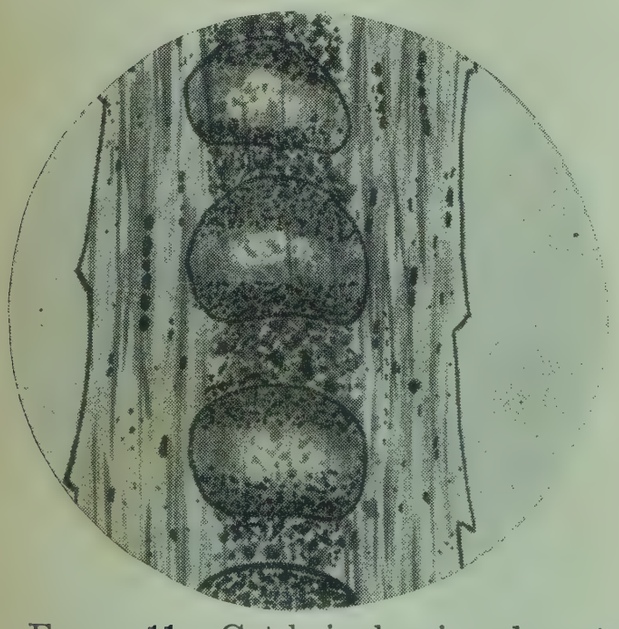

FIGURE 11.—Cat hair showing elongate rows of pigment granules in the cortex, as seen in optical section. Granules below and above focus appear as rows of fuzzy pigment. Medulla consists of air-filled chambers and pigment granules. (Drawn as examined at × 1000).

THE FLATTENED CUTICULAR SCALES OF CAT HAIRS ARE MUCH SMALLER AND FINER THAN THOSE OF RATS AND MICE. In mice the cuticular scales of guard hairs are ovate to crenate and the typically small flattened scales are not found. In rats they are ovate elongate to flattened, THE OVATE ELONGATE SCALES BEING THE MOST POINTED of any of these three.

Fortunately when hairs are treated with 10% NaOH on a microscope slide, the hairs swell and within about half an hour additional diagnostic characters are visible at 400–500 x. After several hours the hairs more or less disintegrate and are no longer usable for study. When the hairs are swollen by NaOH the cuticular scales are enlarged and stand

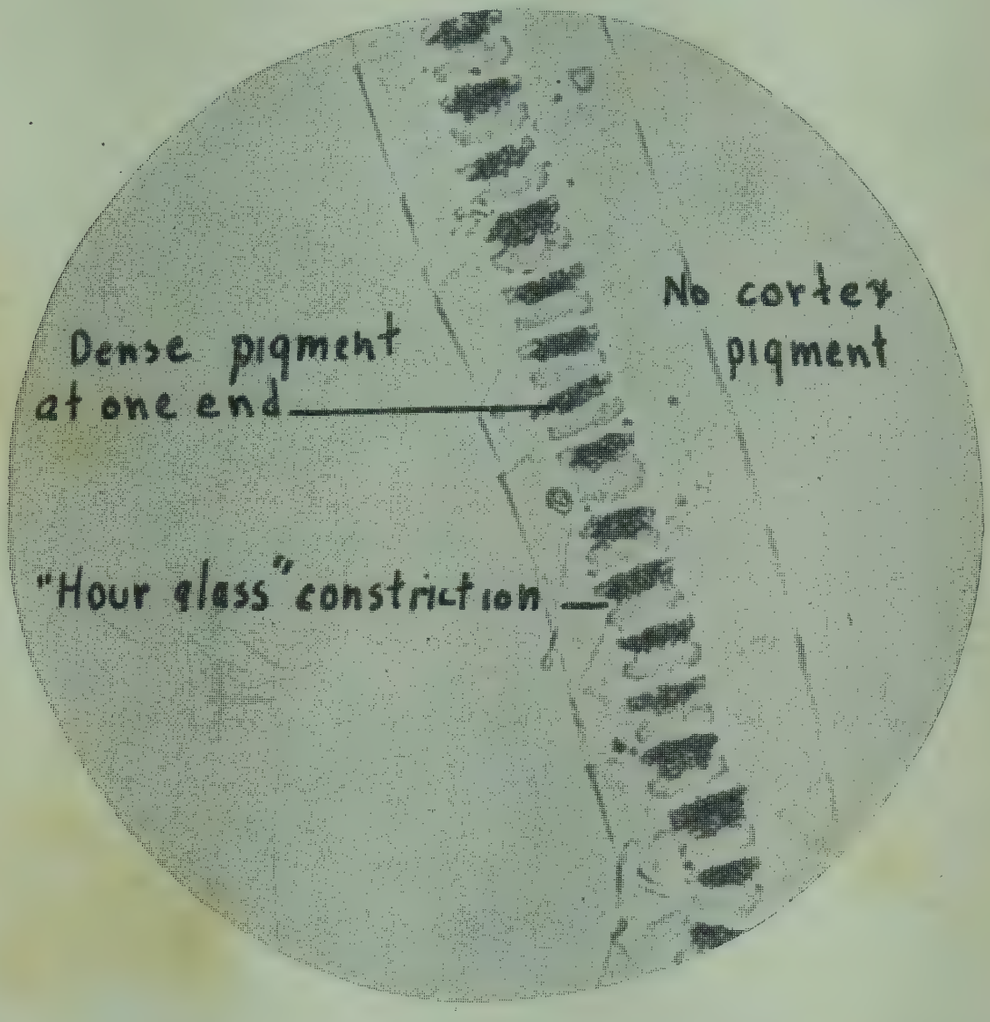

FIGURE 12.—Rodent hair swollen in NaOH, showing medulla pigmentation, constricted medulla shape, and clear cortex.

out against the swollen cortex. Within the cortex the medullary shaft appears as individual segments (also termed cells or chambers) variously shaped and arranged.

The following characters apply to fur hairs treated with NaOH:

RODENT FUR HAIRS: The medulla segments of rodent fur hairs, if pigmented, are compactly pigmented, usually one end of the segment is clear with the compacted pigment at the other although not infrequently both ends of the segment are clear with the compacted pigment in the middle. (See Figure 12.)

Individually, the pigment granules are black or extremely dark brown, although occasionally light brown granules occur. Segments

FIGURE 13.—Cat hair showing the fitting together of adjacent medulla segments and scattered medulla pigmentation.

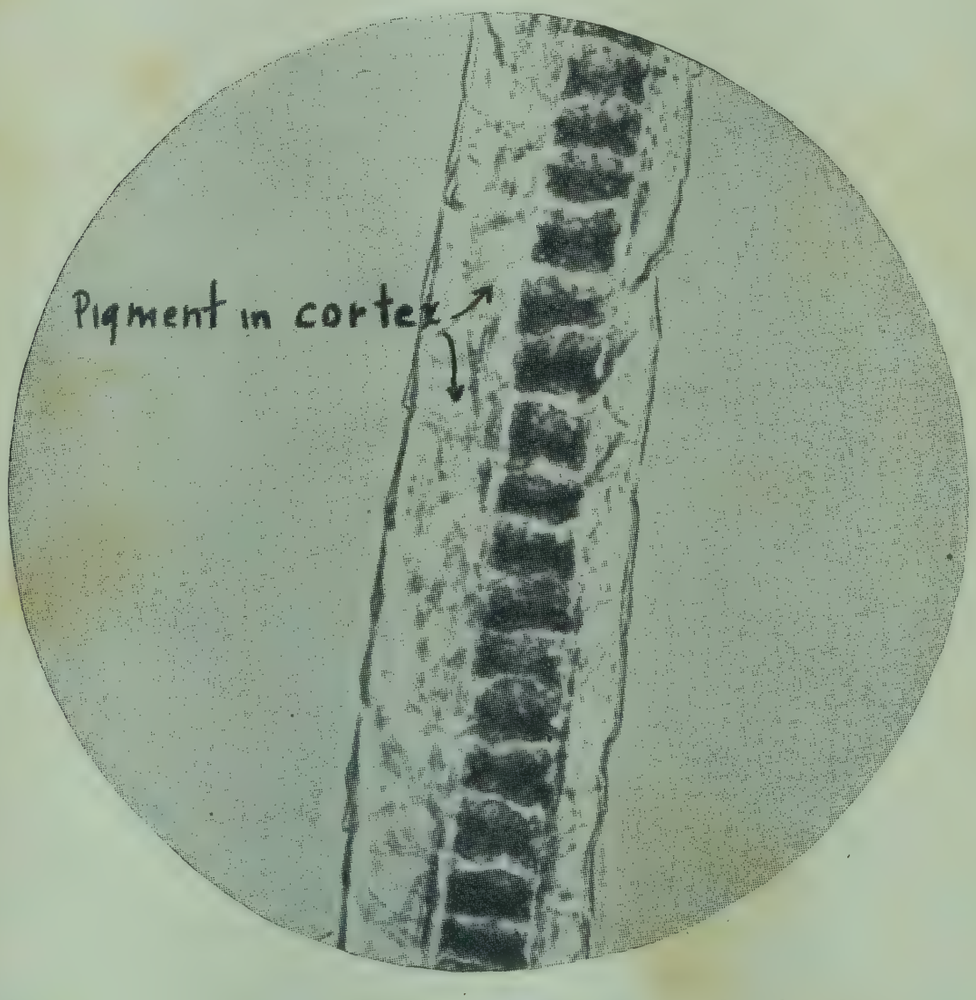

FIGURE 14.—Cat hair showing pigment granules in the cortex.

often are as long (in the direction of hair elongation) or longer than they are wide and have an hour-glass-like constriction in the middle. The segments are relatively far apart when compared to the segments in cat hairs. The cortex as a rule is not heavily pigmented but the pigment granules are rather large and appear compact. The cortex seldom or never appears stained with color except at the extreme tip of the hair. The cortex of rodent hairs has a peculiar clearness which cat hairs rarely if ever show.

CAT FUR HAIRS: In cat fur hairs the segments of the medulla, if colored, are loosely pigmented so that the granules appear scattered. (See Figure 13.)

The pigment granules seldom appear black or dark brown even in black cats. Their usual color is brown or reddish brown. The segments of the medulla, except in Angora cats, whether pigmented or not usually are wider than they are long. These segments are convex on one end and concave on the other. When examined under the microscope they appear as though, if pushed together, adjacent segments would fit similarly to a ball-and-socket joint. They do not touch one another although they are relatively close together and a straight line drawn across the tips of the concave end of one of the segments will in many cases touch the convex end of the adjacent one. Pigment granules often are rather abudant in the cortex of cat hairs. (See Figure 14.) Sometimes the cortex appears stained with color because of dispersed very fine pigment granules. In any case it seldom or never has the clearness of the rodent cortex and this is due in part to faint parallel longitudinal lines which occur in the cortex of the cat hair. RODENT HAIRS SELDOM IF EVER CONTAIN THESE LONGITUDINAL LINES.

GUARD HAIRS (CAT OR RODENT): In addition to the compound medulla of the untreated rodent guard hair and the continuous medulla of the cat guard hair some additional observations on the swollen hairs after treatment with NaOH also appear diagnostic. The medulla of the rodent guard hair breaks up into two or more rows of irregularly shaped blocks that appear to have been fitted closely together until forced apart by the chemical action. (See Figure 15.) These blocks may or may not contain pigment. The medulla of the cat guard hair on the other hand breaks up into thin plates which appear to have been pressed one upon another along the length of the medulla. The individual plates often do not cover the full circumference of the medulla but when they do they appear as a great number of wafer-like discs not unlike the chambered pith of certain plants.

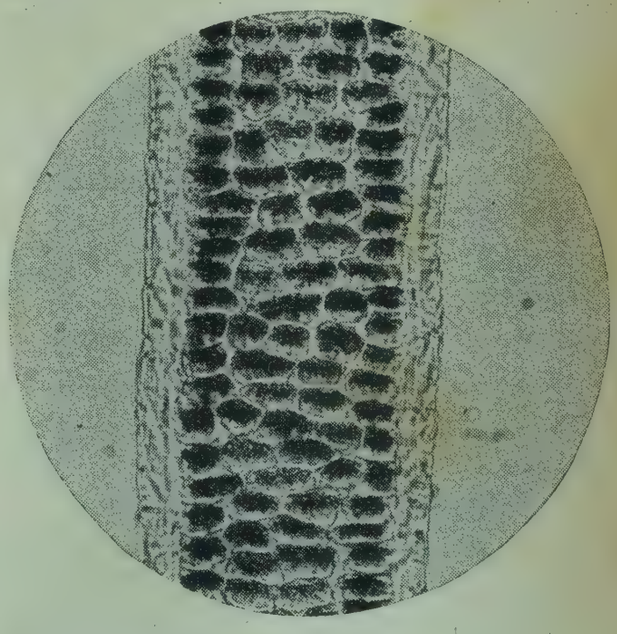

FIGURE 15.—Rodent guard hair treated with NaOH and showing the swollen medulla segments.

For convenience, the characters discussed above are tabulated below:

CHARACTER	RODENT	CAT
Medulla fur hair	Single-rowed, discontinuous	Single-rowed, discontinuous.
Medulla guard hair	Multiple-rowed, discontinuous	Smooth or erose, continuous.
Internodes	Prominent or a few indistinct	Indistinct.
Diameter	Usually smaller	Usually larger.
Cortex size in relation to medulla	Usually narrow	Usually wide.
Cortex pigment	Usually sparsely scattered or absent	Usually abundant.
	None when none in medulla	May be present when medulla is unpigmented.
	Pigment granules in scattered rows	Granules often in dense parallel rows.
Cuticular scales of guard hairs	Ovate, elongate to flat, Some *very* ovate elongate present	Crenate to flat. Some *very* flat present.
In NaOH: Medulla pigment	If present, usually compact, black or dark brown; often localized at one end of segment with other end clear, or at both ends with center clear	If present, composed of loosely scattered granules. Granules brown or reddish; seldom black or dark brown.
Shape of medulla segment	Usually long in direction of hair elongation	Usually wider than long.
	Usually have hour-glass like constriction	Sometimes have hour-glass like constriction.
Distance apart of adjacent medulla segments	Usually relatively far	Usually relatively close.
Contact of adjacent segments	Often squared across	Usually ball-and-socket effect.
Cortex appearance	Usually has a clear gelatin-like appearance	Usually not clear, and with faint parallel, longitudinal lines.
Cortex—in hypochlorite, NaOH, or untreated	Usually not heavily pigmented	Usually abundantly pigmented, with dense pattern of pigment granules arranged in broken parallel rows.

IV. MICROSCOPIC EXAMINATIONS FOR FILTH

A. Treatment of the Filter Papers for Examination Using the Wide-Field Microscope

The type of material trapped off by means of the Wildman trap extraction is dependent upon the nature of the food and the treatment given it. Obviously with floury cereals and bakery products, both starch and bran will be the troublesome factors while in other cases leaf or stem material may be important. Differing amounts of food material are trapped off with the oily layer. The prime objective in making the Wildman trap extraction is to float out the insect and rodent filth while keeping down most of the food material. Too much food material interferes with (1) the filtration and (2) with subsequent microscopic examination.

The suction filtrations can be speeded up by various means. It is usually expeditious (e.g. with M8I) to trap off the oil layer into a beaker where it is allowed to stand and stratify before filtering. If this is done, usually a clear oil layer will form at the surface, followed by an accumulation at the oil-water interface, then beneath this a larger volume of water and fine suspended material, and at the bottom an accumulation of food matter. The advantage gained in permitting these layers to form is that each type of substance will filter more readily alone than when it is in a semi-stable emulsion of the various components. Mineral oil readily filters alone but a mineral oil-water-flour mixture may jam on the filter. Moreover, as will be discussed later, there are ways of speeding certain substances through the papers that would slow up other materials; when they are separated each may be handled separately. In addition, it often is possible to get most of the liquid through before the bulk of the filter-clogging sediment is poured onto the paper so that when the filter finally "jams" the filtration practically is completed.

This last factor is of particular importance with such a method as M2B(1) where small amounts of starch may almost completely clog a filter, yet the amount of starch present, when properly cleared, will present little difficulty when the paper is examined microscopically.

Chemicals may be used to hasten the flow of liquids from the trapped-off solids. For example M4B(1)(a) would present much difficulty if filtration were attempted without the recommended stream of hot water being poured on the paper while the cheese mixture is going through. Similarly, accompanying volumes of hot water can be used with other filth methods except in cases of estimates to be made of the amount of nondescript matter (e.g. M4C(1)(b)) where a given amount of hot water has been prescribed. Water does not particularly help the flow of oil, although hot water may be useful as a means of contributing heat. The usual organic solvents such as $CHCl_3$, CCl_4, ethanol, methanol, petroleum ether, etc., will carry even viscous oils such as castor oil through with them. Chloroform and other heavy solvents work down under the water layer which sometimes is present and start the whole mixture flowing. Alcohol may break an emulsion and start a stubborn filtration or, because of its low viscosity, it may start through a clogged paper and once the flow has begun the other

liquids may follow through. Alcohol, however, sometimes coagulates and hardens an emulsion so that a leathery layer is formed and the filtration must be resumed on a new paper. Acetone is particularly useful for starting the flow of liquid when a suction filtration has stopped because of clogging of the filter paper. Acetone or alcohol also may be used for washing both water and oil from a paper rapidly and for drying it. Concentrated phosphoric acid is very effective for starting a stubborn filtration; when no heat is applied, it will not damage insect fragments or rodent hairs. The phosphoric acid settles down to the filter paper, spreads out, and clears an area through which water or alcohol mixtures can pass. Sodium hydroxide pellets dropped on a filter clear the areas immediately underneath each pellet but should not be used when the examination is to include a search for hairs, since they may be either dissolved or distorted by this alkali.

Filth on a filter paper can be made to stand out with greater contrast to the background if the filter papers are properly treated before being examined with the Greenough-type microscope. Several general purposes should be kept in mind. When the filter paper dries out the only portion which can be examined visually is the immediate surface. To examine further it is necessary to expose more surfaces by a laborious process of digging and probing with needles. Even when this is done it is not feasible to roll particles over so that all sides may be observed. Hairs may be concealed under or within even small pieces of food and thus not be located. Moreover, a dry, dusty filter paper is handled with difficulty so that with almost every movement there is a danger of spilling the material from the paper. For this reason, and there are others to be mentioned later, papers usually should not be allowed to dry out and then cleared but should be treated by a method that will work directly on the oil- and water-soaked debris.

One of the most useful methods when there is little extraneous matter, or when it is necessary to leave water in the mount so that maggots, fly eggs, etc. do not shrink, is to keep the material moistened with WATER OR DILUTE ALCOHOL. In this way the mount remains soft and pliable so that exhibits are removed easily and in quite normal condition. However, this treatment will leave any large residues quite opaque and light spots will be reflected from the irregular wet surface so that at times it is difficult to look through the surface into the underlying material.

A paper cleared in HERTWIG'S SOLUTION (270 gm. chloral hydrate, 60 ml. glycerol, 19 ml. conc. HCl, plus H_2O to make 150 ml.) is practically transparent, but this solution is extremely noxious to work with and seldom is used for routine examinations for this reason. Moreover, hairs and other fairly delicate materials are left with a gelatine-like constituency. Starch is completely gelatinized when warmed in chloral hydrate solutions as are the ordinarily used filter papers, soft insect body parts, etc. If chloral hydrate solutions are used, they may be washed out after the clearing is completed and before the microscopic examination is made. The use of chloral hydrate will be mentioned further for the preparation of mounts for examination under the compound microscope.

MINERAL OIL finds a wide range of uses for microscope mounts and, correctly handled, it should be the most useful agent in preparing

filter papers for examination under the Greenough binocular microscope. In addition to its clearing properties when water is absent, a heavy mineral oil presents a smooth upper surface even when the underlying layers are fairly rough. Thin oil should not be used, for, in addition to partially losing this quality, the filter is not as closely bound to the Petri dish which is used to hold it and so may not lie flat. By far the most consistently successful way of clearing Wildman trap extraction papers in oil has been by the following technique:

Draw air through the paper until practically all of the free water has been removed. Flood with 40 percent alcohol. Draw off this solution and pull air through until the paper is no longer dripping wet but is still supple and damp. Place approximately 2 ml. of heavy mineral oil in the central portion of a Petri dish and gradually lower the paper into the oil from one side so that few or no air bubbles are trapped beneath the paper. Cover the dish and allow it to stand until the water and alcohol have evaporated and until the oil has cleared the paper and material on it. (Note: The material usually will be cleared upon standing overnight.) The paper is wet and soft and remains flat to the glass and oil as the water gradually leaves the upper surface and the oil works in from below. By using a stronger alcohol wash, leaving the cover off, or heating, the oil can be made to penetrate faster but accompanying this more rapid drying there usually will be a crinkling and rolling of the paper which makes any subsequent examination difficult. If speed is essential it is better to add acetone to the filter in the suction funnel and air-dry by drawing air through it and then immediately to clear in oil. Where the dark color of clove oil is unobjectionable it may be used in place of mineral oil and because of its higher refractive index the clearing will be more complete.

B. Treatment of Materials for Examination Using the Compound Microscope

Various materials can be used to mount small objects on microscopic slides depending upon which details are to brought out. To observe or photograph the edges or outline of particles, the material should be mounted in a medium with a distinctly different refractive index. Usually, however, some sort of a compromise is needed so that both the surface and internal structures are apparent and mineral oil, with a refractive index of 1.47–1.48 and the ability to permeate and clear thin dry plant and animal substances, is quite generally used. Mineral oil has added advantages in that objects being examined in mineral oil under the Greenough-type microscope can be transferred directly to it on a microscope slide and studied under the compound microscope. Glycerol is more viscous than mineral oil and is more compatible with certain mounting media for the preparation of permanent exhibits. However, the usual choice between mineral oil and glycerine depends upon other properties of the object. For example, if photographs were being made of a darkly pigmented insect mandible a simple and entirely adequate mounting medium would be mineral oil, but to plunge a maggot into mineral oil would result in an opaque blur because of the immiscibility of the moisture in the maggot and the oil. The mandible could be air-dried and plunged directly into the oil. Air drying of the maggot would result in a shrunken twisted object valueless as an

exhibit, and to dehydrate the maggot gradually and then mount in oil would not only be time-consuming but might so completely clear the object that for photographic purposes it would lack "body"; a 50-percent glycerine in water solution might be better for this kind of a subject.

Solutions of chloral hydrate are extremely useful for the clearing of microscopic objects. Chloral hydrate clears about the same materials that will clear in alkali but for many tissues it is more readily handled and controlled. Eight parts chloral hydrate in 5 parts water (W/V) often is recommended but by substituting glycerine for some of the water a less volatile mixture is obtained which can itself be used as a mounting fluid. A little experience with either of these mixtures or with Hertwig's solution on cereal tissue, muscle, etc., will demonstrate how effective this clearing agent is. Hertwig's solution is effective even at low temperatures and larvae, for example, will be reduced to gelatinous sacks if allowed to remain in it for several weeks. It is most useful when the tissues to be cleared of interfering substances are more resistant than the remainder of the material.

Temporary mounts with glycerine or mineral oil as a mounting medium can be converted to more permanent mounts by ringing the coverglass with an appropriate cement such as Canada balsam in xylol, Bell's Microscopic Cement, Murrayite, Clearcol, etc.

It should be remembered that to take photographs or make observations using a compound microscope and transmitted light the subject must be surrounded by a transparent medium and unless an immersion objective is being used a coverslip must be used so that the interface between the mount and the air is flat. If this surface is not flat then the image of the object will be distorted. When reflected light is being utilized the presence or absence of a coverglass is determined by what is to be studied, (e.g., surface irregularities, outline, internal structure, color).

Discussions of the procedures to be used for dehydrating tissues, clearing in xylol and mounting in balsam may be found in almost any textbook of microscopy but, for convenience, the steps will be summarized here. As noted earlier, dry material can be mounted in mineral oil "as is." Similarly it can be mounted in balsam except that as an added precaution to remove air and assure a thorough penetration by viscous balsam the tissue should first be passed through xylol. By relaxing the tissue it is possible to restore it to somewhat of its original shape. For example, a flour infested with larvae and then fumigated will contain not round turgid larvae but shriveled dried out specimens. These larvae can be soaked and then warmed in dilute (1–3 percent) sodium hydroxide solution until they have relaxed and swollen to approximately their original shape.

The usual procedure for dehydrating tissues consists of passing the tissue successively through 20, 40, 75, 85, and 95 percent alcohol, two changes of absolute alcohol, two changes of xylol, and then balsam. The amount of time in each solution of course is proportional to the size of the tissue and it should be long enough to allow an equilibrium to become established in each solution so that the dehydration is gradually accomplished and tissue shrinkage is kept at a minimum.

V. USE OF THE MICROSCOPE

To cover, even in a sketchy manner, the theory of the formation of the microscope image would involve pages of discussion centering around several controversial issues. Fundamentally, however, there is a general agreement as to the practical means to employ to obtain an image with certain qualities. In regulatory filth work we usually are *not* working for maximum resolution. It is well to keep this in mind, for much has been written on the use of the microscope and much of this work has been concerned with the adjustment of conditions to fulfill this end.

Among all of the controversial optical subjects there is agreement on one fundamental point, the ability to resolve two objects, that is, to see them as distinct images, depends upon the size of the angle formed by perpendiculars from these objects to the eye. (See Figure 16)

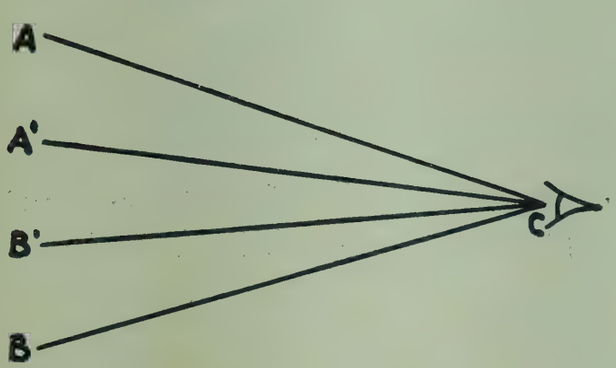

FIGURE 16.—Angular separation of two points with respect to the eyes.

FIGURE 17.—Angle view of a point as seen when the objective is filled with light.

Two points an inch apart and 1 foot from the eye make a relatively large angle and are easily separated. (ACB) A hundred yards away the angle has shrunken to a fraction of a degree. (A′CB′) The microscope is simply a means of increasing the size of that angle, or, to put it differently, the microscope is a means of placing the eye closer to the object being viewed. With the ordinary 10x achromatic objective this object distance is approximately 7 mm. With the 10x apochromatic objective this distance is approximately 5 mm. With an oil immersion objective the object distance is zero (0), and optically the object is within the first lens element. The image produced by the objective is projected by the eyepiece so that it is visible to the eye, and inasmuch as the image produced by the objective contains details too small to be seen with the unaided eye, the eyepiece commonly is designed to add around 5–15x magnification.

To obtain the maximum resolution it is essential that light coming from the object and having the greatest angle from the optical axis, be caught by the objective. If a diaphragm were placed in front of the objective the resolution would be diminished. In other words, the front lens of the objective should be filled with light. (See Figure 17.) To fill with light all but the lowest power objectives it is necessary to use a condenser under the object. This condenser often is incorrectly used for

many purposes, but it is designed primarily to project through a transparent object a cone of light sufficiently large to completely fill the front lens of the objective with light.

The quality of the image is also determined by the degree of contrast between markings on the object and the general background. In microscope manufacture many precautions are taken to keep the contrast high. Among other things, stray light is eliminated so that it does not fill the visual field and produce a greying of the image. The condenser should be open only enough to fill the front lens, since light striking from the edges of the objective will be reflected within the instrument and reduce the sharpness of the image.

As indicated earlier, factors other than maximum resolution are important, and it is often more simple to switch to a higher power objective. Where thick mounts are involved, an increased depth of focus may be needed. While the resolution is proportional to the aperture of the objective the depth of focus is inversely proportional to the size of the aperture. (See Figure 18.) The simplest way to reduce the

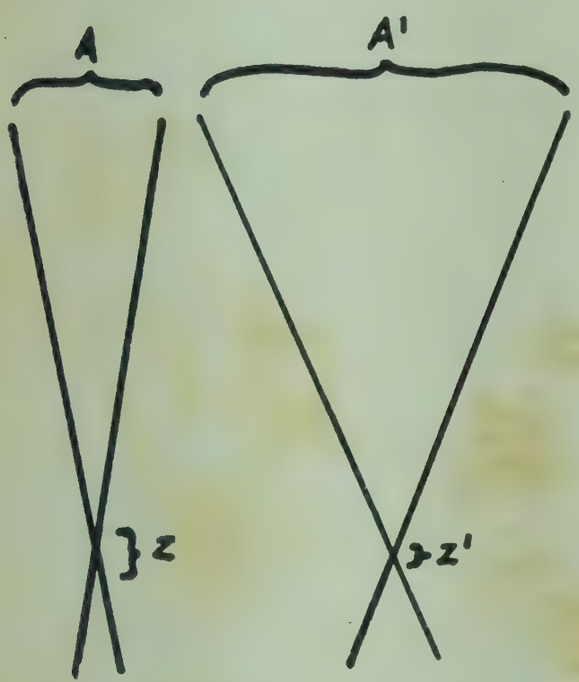

FIGURE 18.—Depth of focus. Small aperture A gives large depth of focus; large aperture A′ gives shallow depth of focus.

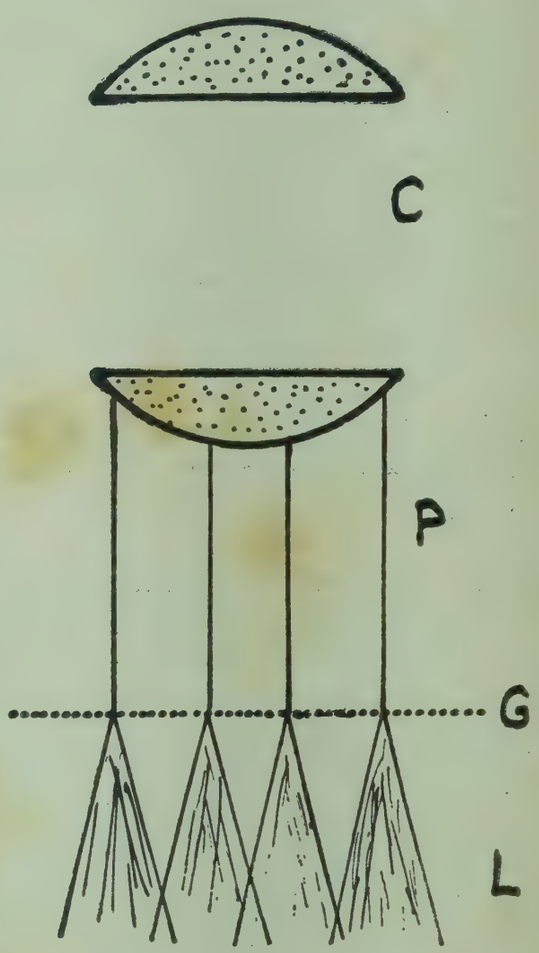

FIGURE 19.—Illumination by parallel light from a ground glass. C, Condenser forming parallel rays; P, Parallel rays; G, Ground glass; L, Light rays emitted from a ground surface.

size of the cone of light entering the objective is to stop down the iris diaphragm of the condenser. Such a practice is often utilized in mold counting where it enables the observer to scan the deep mount with less focusing than otherwise would be necessary.

Up to this point nothing has been said about the source of illumination. The condenser is designed to focus parallel light and should be so

used. Some observers maintain that no oblique rays should enter the condenser and while they are correct in theory, the practical effect of such light is very slight. For practical purposes it is necessary only to provide approximately parallel light of the correct area and intensity. Many sources have been used but so far as standardized regulatory work is concerned a flat filament bulb focused at infinity with a condensing lens system, but actually illuminating a ground glass, is preferred. (See Figure 19.) It has been found that when parallel rays from a flat filament impinge upon the ground glass the pattern of light emitted from the glass is scattered, but not to the extent that it would be scattered if diffused light were used as an illuminant. To obtain light most of which is parallel the illuminated ground glass should be at least 9–10″ from the microscope mirror.

By adjusting the position of the condenser the image of the ground glass should be projected into the plane of the object. If the pattern of light is annoying, the condenser can be raised slightly and the sandy background eliminated. In this position the condenser system is utilized to best advantage with the rest of the microscope.

Most modern microscopes are provided with a few simple adjustments which must be made by the observer and which, when correctly made, will provide for better results and easier use. On most lamps and on the lower side of the condenser there are holders for light filters. Most people prefer a blue-white color, but other colors will prove useful under some conditions. For example, green will give more contrast to red objects; yellow may be preferred with blue. Two important adjustments should be made at the eyepiece end of the binocular body tube to fit the microscope to the individual eyes. The distance between the binocular eyepieces should be adjusted to the observer's interpupilary distance and the movable eyepiece should be placed at such a height that both eyes see a focused image at the same time. When a 1.30 or 1.40 numerical aperture condenser is brought in focus and then the iris adjusted to either the correct aperture or a comfortable light intensity with all but high power objectives, it will be found that the entire field is not illuminated, the edges remaining dark. The condenser should not be lowered to remedy this situation, but instead, the top element of the condenser should be removed to reduce the aperture to around .40–.75, depending upon the manufacturer and the model involved. (See Figure 20.) Except under certain specific instances the flat side of the mirror should be used, the mirror being used solely to bend the light rays up into the microscope.

FIGURE 20.—Separation of the condenser. *A*, Top element; *B*, Lower element.

As noted earlier, mold counting is usually done with an increased depth of focus. The intensity of illumination should be varied by opening the iris diaphragm to obtain sufficient penetration to see through dense clumps of tissue, and by closing it to prevent the observer from "looking through" fine pieces of mold.

At higher magnifications than the 100x–200x used in mold counting, the illumination must be more closely controlled. For example, when examinations are made for yeasts at 400x–500x the condenser must be focused and the diaphragm opened so that the objective is used at, or very close to, full aperture. In addition, the intensity of illumination should be carefully adjusted so that the yeasts are neither lost in an overly bright background nor overlooked if there is a lack of contrast in a cloudy dark field.

The Greenough-type stereoscopic microscope differs in several fundamental respects from the compound microscope. Through the use of erecting prisms within the body tube, an erect image is seen. Each eye views the object through an optical system that is independent of the other eye and which focuses upon the image from a slightly different angle. Moreover, the paths of light through the microscope are converging and true stereoscopic vision is obtained. This system is applied to rather ordinary-sized fields by some European manufacturers and, in the past, one manufacturer in this country made such an instrument capable of rather high magnification (90x–100x) while still retaining good resolving power. Such equipment has been supplanted by the so-called "wide field" instruments, which now are commonly used for the detection of filth. With a wide-field stereoscopic instrument, fields with a diameter of 7–10 mm. may be examined at 25–30x. By using a transparent background and preferably transmitted light, good definition can be secured at 40x–50x, but as use is made of powers beyond about 50x, the magnification is empty (i.e., increased magnification without increased resolving power). For studying fine details above 45–50x, objects should be cleared and studied under the compound microscope.

When using the Greenough-type microscope, sufficient, even, and correctly colored light is important. There is little danger of obscuring details by introducing a haze into the optical system, and the upper limits of intensity are best determined by eye comfort. For searching over a flat surface for small, up to 1 mm., particles of hairs, insects, bran, seeds, etc., the light should be focused into the field from one direction so as to avoid the soft effect obtained with diffused light. The intense illumination should cover, without shadows, the entire field of view. The most satisfactory type of illuminator consists of a low-voltage bulb set in an adjustable mount in an elliptical mirror so that diverging, parallel, or converging light is obtainable. The lamp should be mounted on a jointed arm to permit illumination from any angle. By use of the focusing mount and jointed arm mounting it is possible to keep the lamp well outside the field of observation. Such a low-voltage lamp should be provided with an adjustable transformer so that the observer can adjust the light intensity to the actual work at hand. As will be found by actual practice, the light intensity must be changed depending upon the color of the objects being searched for, color of the background, and magnification used.

VI. OPTICAL CRYSTALLOGRAPHY

In the examination of foods and drugs to determine their composition it is often difficult to identify certain of the ingredients by purely chemical methods. Such methods usually require long, drawn-out procedures and an appreciable amount of the sample. Conservation of both time and materials can be accomplished by the application of microscopic methods. By the examination of diagnostic tissue elements and cell contents, the various plant ingredients of a capsule or tablet can be identified by microscopic methods. Similarly, diluents in such mixtures can also be identified.

Crystalline ingredients, often present in too small an amount to be handled efficiently by chemical methods, can be identified by their optical crystallographic properties. Not infrequently the consumer has noted in a food product hard, colorless fragments which he suspects are glass particles. The occurrence of such particles is not uncommon in seafoods, particularly shrimp, sardines, or caviar. A quick and accurate method of identification of these foreign particles where time and the amount of material at hand are important factors is available through optical crystallographic methods. By the use of the polarizing microscope and the immersion method the foreign material in seafood is invariably identified as ammonium magnesium phosphate or "struvite." Occasionally samples of cheese contain embedded in pockets small amounts of glass-like crystalline material. Such material is usually found to be crystallized milk sugar, but occasionally it has been identified as calcium tartrate.

These few examples of the use of optical crystallographic techniques in the analysis of foods and drugs indicate the value of such methods in regulatory work. There is discussed below in considerable detail fundamental principles that will be of value to the analyst interested in this field.

Crystallography is concerned with the structure and related properties of solid substances the atoms of which are in orderly geometrical arrangement. This internal arrangement is the same throughout the entire extent of the crystal, the atoms comprising the molecule being arranged in a definite and regular order. The nature of this arrangement is determined by investigations of the space lattice, based principally on the diffraction of X-rays by the various planes of atoms comprising the molecule, the diffraction patterns revealed being indicative of the internal structure.

When a crystal grows freely in all directions, the forms shown, represented by faces of different shapes and sizes, constitute the habit of the crystal. During the process of crystallization, various crystals may assume shapes depending upon conditions. As a result of the effect of these conditions the habit may be modified, producing distorted crystals, with the suppression of some faces and the increased development of others. In the formation of crystals, a substance passes from the state of a fluid to that of a solid under conditions favorable to the natural adjustment of the atoms and significant for that substance. In other words, in this orderly adjustment there is the same arrangement of atoms of one kind about any one atom as about any other one of

the same kind within the crystal. In case this natural adjustment is attained, the interatomic forces are in equilibrium. Investigations of the arrangement of the atoms in the molecule or the space lattice are beyond the scope of the polarizing microscope.

Examinations of crystalline material by the polarizing microscope reveal optical properties that are dependent upon the internal structure of the crystal and are not affected by the external form. Even when there is no external evidence of crystalline character, that is, geometrical form, the miscroscopic-crystallographic study alone may yield valuable information. In this examination the actual solid phase may be investigated directly rather than merely the ions which the crystal yields when dissolved. These methods have been applied to minerals for many years and the same technic can be applied to synthetic crystalline substances.

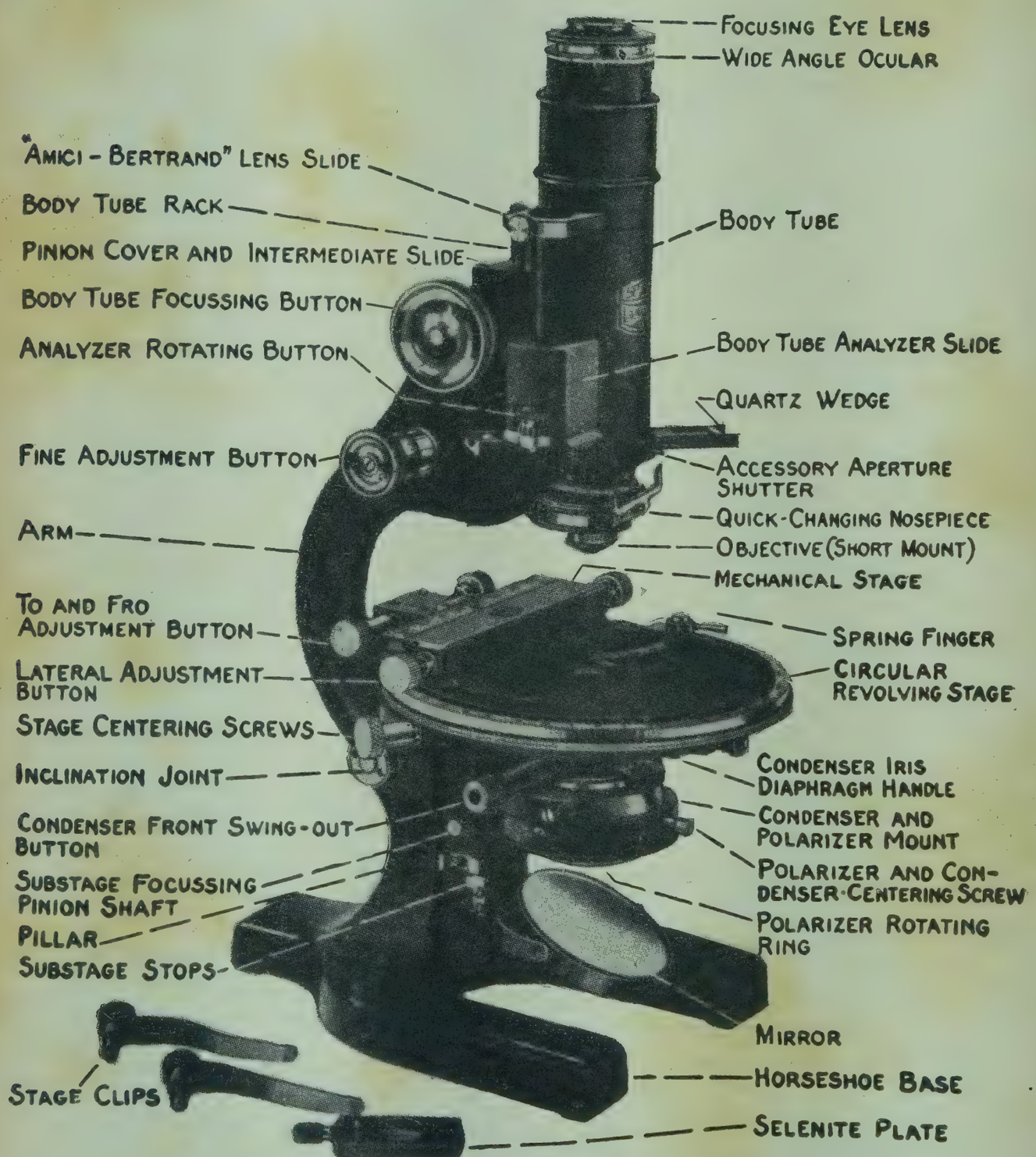

Figure 21.—A student model petrographic microscope with a mechanical stage added.

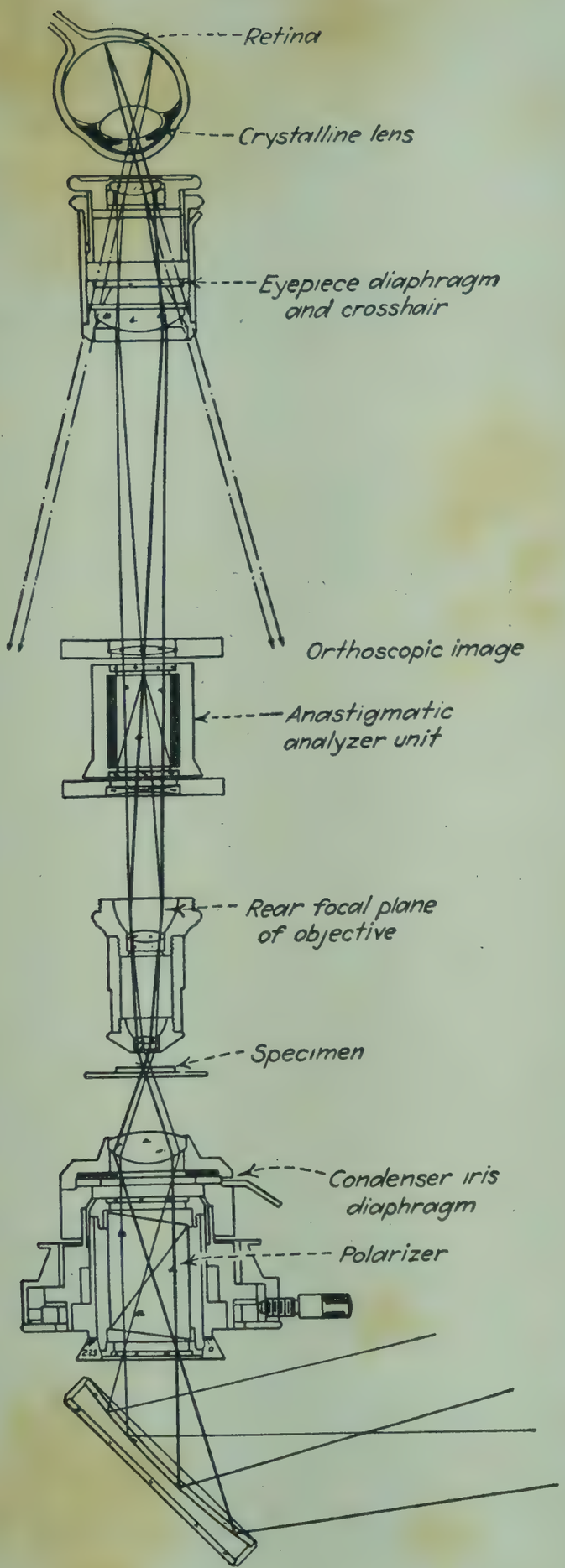

FIGURE 22.—A diagram illustrating the path of light through the microscope for ordinary observation with crossed nicols.

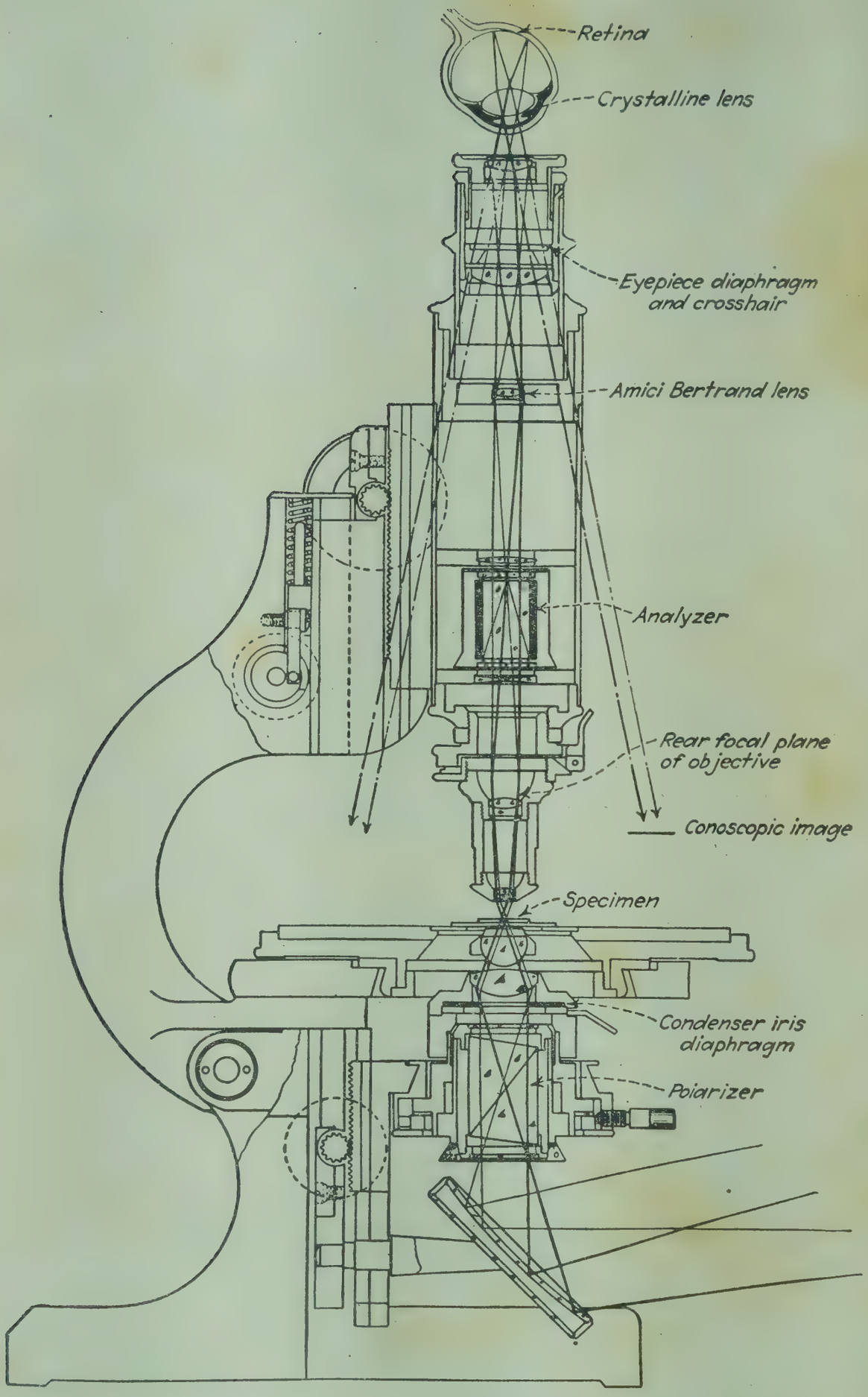

FIGURE 23.—A sectional view of the polarizing microscope as a conoscope for the study of interference figures and also for high magnifications.

The polarizing microscope with its various parts is illustrated in Figure 21. Figure 22 diagramatically illustrates the path of light through the microscope for ordinary observations with crossed nicols. Figure 23 shows a sectional view of the polarizing microscope arranged as a conoscope for the study of interference figures.

For practical purposes in the use of the polarizing microscope it will be appropriate to consider light as a form of energy consisting of wave motions in the ether. On this assumption research has shown that these waves are very short and those of different wave length (periods of vibration) produce different colors. That is, violet light and red light in the spectrum will have different wave lengths. The light from a luminous flame owes its brightness to the large number of incandescent particles it contains. These individual particles send out light waves in all directions, these being reflected and refracted by various objects in their paths. Many of these waves may combine or pile up, thus increasing their intensity, while certain other waves may neutralize each other. Dropping a rubber ball into a quiet pool of water will send out a series of waves spreading in all directions. If a large number of these balls is dropped into the water a series of waves would originate from each ball causing a heterogenous mixture of waves. It would therefore be difficult to determine the relationship between any one ball and the particular set of waves originating from it. Ordinary light is analogous to the conditions produced when numerous balls have been dropped on the water surface. Parallel polarized light (crossed nicols) is analogous to the conditions produced by the one ball. Therefore, polarized light can be defined as light whose wave vibrations move in one plane. Ordinary light can be polarized in several ways. The method usually employed in microscopic crystallography is polarization by a calcite prism, usually known as a nicol prism.

A. DOUBLE REFRACTION (BIREFRINGENCE)

Crystalline substances, other than those belonging to the isometric system, not only refract light but divide the beam into two rays which travel at different velocities. These two rays vibrate at an angle which is approximately 90°. Calcite, a very pure form of calcium carbonate, will resolve a beam of light into two rays. If a piece of calcite is placed over an object, two images of the object are produced. In other words, if a single beam of light is sent through a prism of calcite, two beams will emerge on the opposite side. Each of these beams is plane polarized, the plane of each being approximately at right angles to the other. A piece of calcite suitably cut and recemented will remove one of these rays by total reflection. Only one beam emerges and this is plane polarized light (light vibrating in one plane only). A piece of calcite thus cut and recemented is usually spoken of as nicol prism, or simply a nicol.

In Figure 24 is illustrated diagramatically the passage of a beam of light through a section of nicol prism. A ray of light, XY, upon striking the lower surface of the nicol is resolved into two rays vibrating in perpendicular planes. Since the refractive index for the ordinary ray in calcite is 1.658 and the index of refraction of the balsam layer cementing the two sections is about 1.54, the ordinary ray, YZK, is so bent that it is totally reflected at the contact of the calcite with

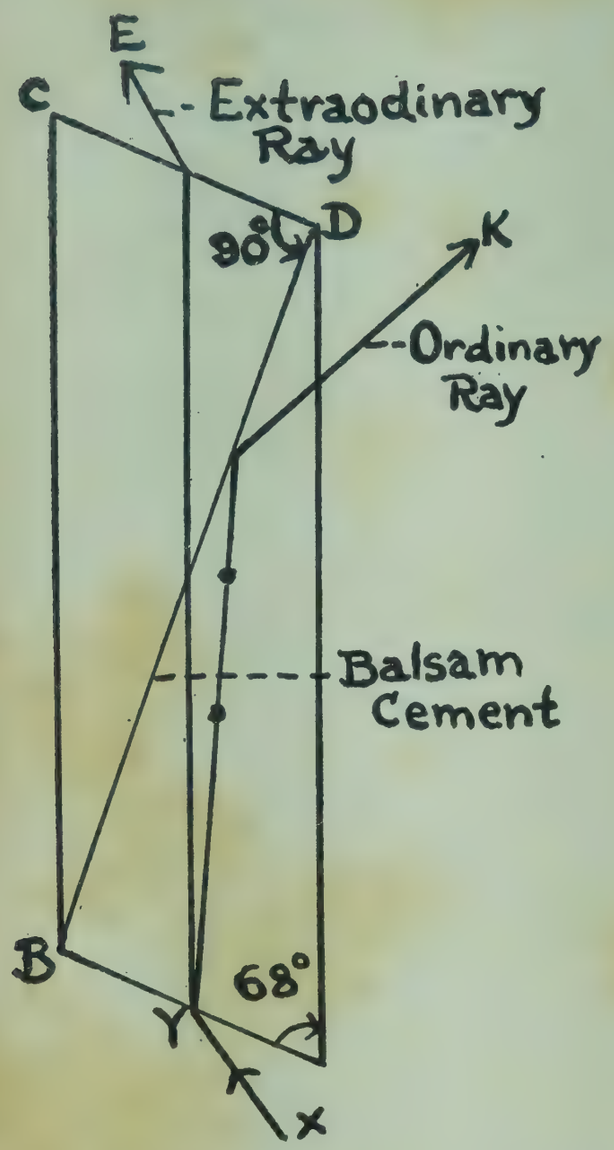

FIGURE 24.—Illustrating transmitted light through nicol prism.

the balsam and passes into the walls of the prism where it is absorbed. The extraordinary ray for calcite has a refractive index of 1.486 and rays traveling in intermediate positions between the ray with the minimum index and the optic axis have refractive indices lying intermediate between 1.658 and 1.486. The extraordinary rays moving parallel or nearly so to the long direction of the prism have indices approximating 1.54, the value for balsam. The extraordinary rays travel through the balsam layer unimpeded and emerge from the upper surface of the prism as plane polarized light.

Suppose, for example, an isometric substance like sodium chloride (C–2) is examined between the crossed nicols. Since the crystal does not exhibit double refraction the light emerging from the polarizer is not altered and therefore enters the analyzer just as it would if the crystal were not present. No matter how the crystal is oriented, the effect, or rather, lack of effect, on the light is the same. All of it is totally reflected internally by the analyzer, and the observer sees no light at all emerging from the analyzer. On the other hand crystals of corn sugar, or dextrose hydrate as represented in C–6 (doubly refracting substances) act on the light emerging from the polarizer just as the analyzer would. If the plane of vibration of the polarizer and the crystals are parallel the light emerges from the crystals with practically no diminution in brightness and with its plane of polarization the same as if the crystals were not present. This light is then lost in the analyzer by total internal reflection, again as if the crystal were not present, and the observer sees no light emerging from the analyzer. If the crystal is rotated somewhat out of its position of parallelism, the light beam coming from the polarizer is doubly refracted anew into two beams with planes of polarization at right angles to each other. There is no arrangement in the crystal to absorb either of these beams as there is in the nicols. Consequently both beams enter the analyzer. That small part of the light which is vibrating parallel to the plane of the analyzer is transmitted and the observer sees the crystal faintly. As the crystal is rotated, more and more light is transmitted until the 45° position is reached. At this position the crystal is the brightest. As the rotation becomes greater and greater, less and less light is transmitted, and the crystal appears less and less bright until the 90° position is reached, in which no light is transmitted and the effect to the observer is the same as at the beginning of the rotation.

When the analyzer and polarizer are parallel, the polarized ray passes through the analyzer without double refraction and comes out without any appreciable loss of brightness and without any change in the position of the plane of polarization. As the analyzer is rotated, double refraction takes place, and none of the ray is lost by total reflection in the analyzer. By rotating the analyzer more and more, the light lost by internal reflection increases in amount, and the light emerging becomes less and less. There are two positions of the nicols which are important in optical crystallography:

(a) The parallel position, in which practically all the light leaving the polarizer (lower nicol) is transmitted through the analyzer.

(b) The cross nicol position, in which none of the light emerging from the polarizer is transmitted through the analyzer.

On the basis of the phenomena shown by crystals when rotated between crossed nicols, there are two great classes of substances, the singly refracting and the doubly refracting, or, in terms of their effects, those which remain dark during rotation and those which alternately light up and grow dark during rotation. This test is fundamentally one of the most important in optical crystallography. Not only does it give a ready method for distinguishing two major groups of crystals, but it has a very definite bearing on the refractive indices of a given crystal, as will be shown subsequently.

B. Refraction of Light and Refractive Indices

An elementary principle of physics may be illustrated by placing a glass rod in a beaker of water. When this is done the rod appears to be bent where it enters the water. Light behaves similarly when passing obliquely from one medium to another. The ray of light travels with different velocities in different media and thus because of "bending" or refraction undergoes a change in direction. This phenomenon is illustrated in Figure 25. The ray AB in air strikes the surface of the water at B and upon entering the water does not continue in a straight line but is refracted or bent towards the normal RS, since the velocity of light is less in water than it is in air. A ratio can be established between the velocities of light in the two media. If the sine of the angle of incidence i is divided by the sine of the angle of refraction, r, a numerical figure is obtained and this is known as the INDEX OF REFRACTION, represented by the symbol n. Thus the index of refraction of water in terms of air is 1.333; of common salt (C–2) 1.544, and of the diamond 2.42. It is therefore apparent that the velocity of light in a given medium is proportional to the reciprocal of its index of refraction. Hence, the larger the index, the slower the velocity, and vice versa.

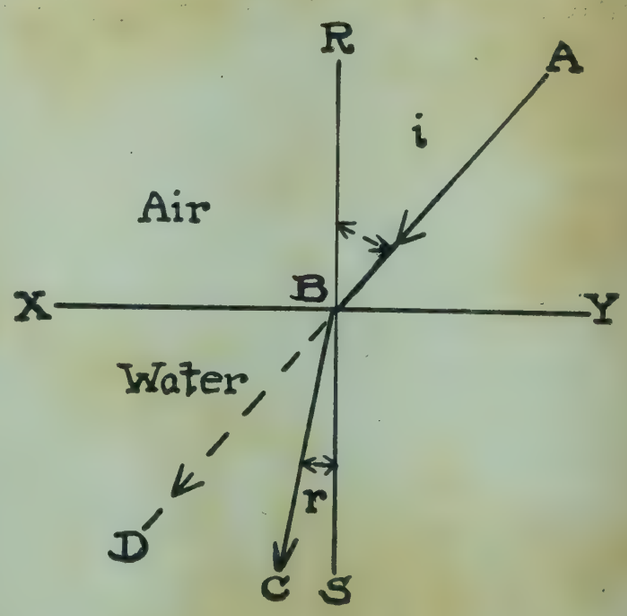

FIGURE 25.—Illustrating the principle of refraction.

For the determination of the refractive indices of crystalline substances, the use of monochromatic light is preferable to white light. It may be readily observed that when white light is passed through a prism it is split up into its component colors, that is, a spectrum is formed. The rays from the red end of the spectrum have a greater velocity than those from the violet end. In the case of monochromatic light, the light is of one wave length, such as the yellow light of sodium which has a wave length of 589 mμ. The indices (or index) of refraction of a crystalline substance therefore will vary with the wave length of light used and be different at the two ends of the spectrum. This difference in light velocity at the extreme ends of the spectrum is called DISPERSION. The difference in the indices between opposite ends of the spectrum indicates the strength of dispersion. The dispersion of glass (C–15) is much lower than that of a diamond.

Crystalline substances can be classified into ISOTROPIC OR ANISOTROPIC substances, depending on how light travels in them. ISOTROPIC SUBSTANCES are those in which the light travels with the same velocity in all directions. Isometric substances (cubic) such as sodium chloride (C–2) are isotropic. ANISOTROPIC SUBSTANCES are those in which the velocity of light varies with the direction of transmission. Anisotropic substances can be further divided into UNIAXIAL and BIAXIAL groups, which will be discussed later.

In the study of crystalline substances with the polarzing microscope, isometric crystals, which are singly refracting, will have one and only one refractive index, usually denoted by n. Substances crystallizing in the tetragonal and hexagonal crystal systems are doubly refracting and have two significant refractive indices, usually designated as n_ϵ and n_ω. Orthorhombic, monoclinic, and triclinic substances have three significant refractive indices, usually designated as n_α, n_β, and n_γ. Lactose hydrate (milk sugar) represented by slide C–16, illustrates the monoclinic system and has three significant refractive indices, $n_\alpha = 1.517$, $n_\beta = 1.542$, and $n_\gamma = 1.550$. The method for the determination of these indices will be discussed later.

C. INTERFERENCE OF LIGHT

A discussion of the principles underlying the interference of light waves will be appropriate before it will be possible to interpret some of the phenomena observed in uniaxial and biaxial crystals. When a rubber ball is dropped onto the clear surface of a pond it will be first observed that the ball has an up-and-down motion, without horizontal displacement. If the water moved horizontally, the ball would move horizontally with it, and the fact that no such horizontal movement takes place is evidence that the wave motion is simply a progressive up-and-down movement of the surface of the water. At the spot where the ball strikes the water surface, there is at first a depression, and immediately afterward there is a rise. This up-and-down movement is transmitted to other objects, and ultimately carried farther and farther from the initial source of disturbance, forming waves.

In Figure 26, AB is the level of an undisturbed surface of the water, and *abcdefg* is the same surface in wave motion. The highest points of the waves, such as *b* and *f*, are called CRESTS. The lowest points, such as *d*, are called TROUGHS. The distance *ae* or that between any two points

having the same level and corresponding directions of motion, is called a WAVE LENGTH. The distance *ob*, that is, half the vertical distance from a crest to a trough, is called the AMPLITUDE OF VIBRATION. WAVE LENGTHS determine the color of the light. Those rays which give our eyes the sensation of red are the longest waves of the spectrum and those giving the sensation of violet, the shortest. White light is a mixture of all wave lengths from that of violet to that of red.

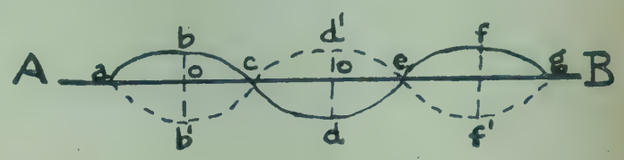

FIGURE 26.—Illustrating interference of light waves.

When two waves of light of the same length are superimposed so that every point in one is in the same phase as corresponding points in the other, the wave length will remain unchanged. However, the forces acting vertically will be increased, and the amplitude of the new wave will be correspondingly greater and the intensity of the light therefore increased. Conversely, if two waves of the same length are superimposed so that every point in one is in the phase opposite to that of the corresponding points in the other, the vertical forces will neutralize each other, and the amplitude of the new wave will be zero. There will really be no wave at all and darkness will be the result. Such materials as extremely thin films (soap bubbles) are good examples. When these are examined in monochromatic light, a series of alternate bright and dark rings will be observed, the dark rings resulting from the interference of light. If white light is used in place of monochromatic light, only certain wave lengths interfere in given positions. The light reaching the eye is highly colored because certain waves have been removed from the original white light. Instead of dark rings, as in the case of monochromatic light, a series of highly colored rings results. This same phenomenon is observed when a cone of light is sent through a doubly refracting substance. This will be more fully explained when interference figures are described later.

D. UNIAXIALITY AND BIAXIALITY

1. Uniaxial Substances.

Reference has already been made briefly to the division of anisotropic substances into two groups, the uniaxial and biaxial, depending upon whether the crystalline substance possesses one or two isotropic directions. These isotropic directions are designated OPTIC AXES. These are the directions in which light can travel through a doubly refractive substance without exhibiting the usual phenomena of extinction and retardation (see discussion under "Compensators"). Those crystalline substances with one isotropic direction possess two significant indices of refraction, including substances crystallizing in the hexagonal and tetragonal systems. These two refractive indices may be designated as n_ϵ and n_ω.

By means of the polarizing microscope it can be determined whether a substance is uniaxial or biaxial. This is done by converting the microscope into a conoscope. Such an instrument is essentially a wide-angle telescope, in special form, and devised for the study of interference figures. To convert the chemical microscope into a conoscope,

the converging lens of the condenser is raised by means of the substage as close to the microscope slide as possible and by using a high power objective (4 mm.), removing the ocular, crossing the nicols, and observing the interference figure produced by the crystal fragment being examined. In the ideal case for a uniaxial substance, when the cone of light, under conditions as just described, is passed through the crystal fragment, the rays at the center of the cone will strike the substance at right angles and will pass through without double refraction. All other rays, however, will be doubly refracted more and more as their angular distance from the axis of the cone is made greater and greater. At any given distance from the axis the difference of path due to the double refraction will be the same, and the visible result, in the ideal case, will be a series of concentric circles showing a succession of colors in the Newton rings. The crossed nicols cut out all light vibrations parallel to their planes, and consequently the series of circles is broken by a black cross (Figure 27*A*). The center of this

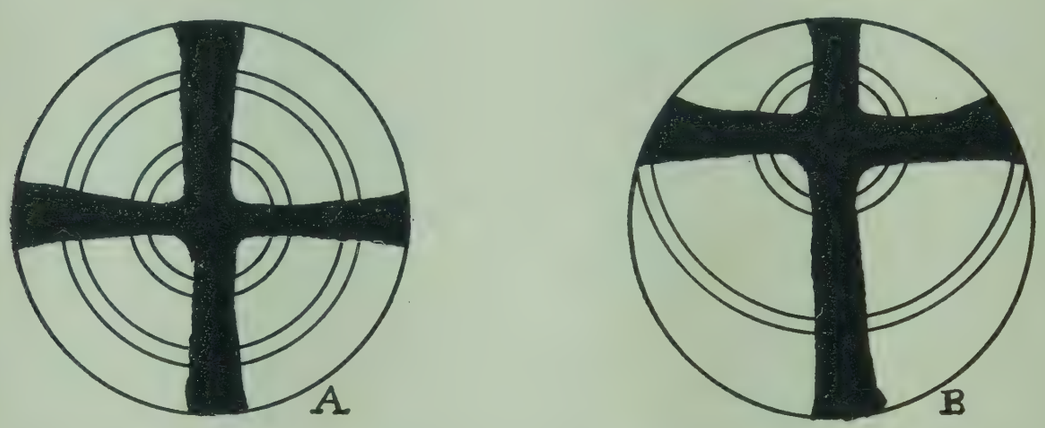

FIGURE 27.—Uniaxial interference figures. *A*, Optic axis vertical; *B*, Optic axis inclined.

cross is coincident with the center of the rings and is on the optic axis. If the axis of the cone of light and the optic axis are parallel, the center of the cross is in the center of the field of the microscope. As the stage of the microscope is revolved, no change whatever takes place. If the optic axis and the axis of the cone are slightly inclined to each other, the center of the cross will be slightly outside the center of the microscope field, and will describe a circle around the center of the field as the stage is rotated (Figure 27*B*).

As already indicated, uniaxial substances have two refractive indices, one of the rays vibrating parallel to the optic axis and another at right angles to this direction. From the indices obtained it can be determined whether the substance is optically positive or negative. If $n_\epsilon > n_\omega$ in a uniaxial substance, the material is optically positive; if the reverse is true, negative.

2. Biaxial Substances.

If a biaxial crystal is examined in convergent polarized light with crossed nicols (conoscope as described above), the phenomena are more complicated than in the case of uniaxial interference figures. If interference can be obtained, the following three types may be encountered, not all of them necessarily being found on the same substance.

(a) Crystal Fragments Perpendicular to the Acute Bisectrix. For purposes of clarification, the acute bisectrix is defined as the median line that bisects the acute angle formed by the emergence of the optic axes. The latter appear as "eye spots" in the interference figure (Figure 28). Crystal fragments perpendicular to the acute bisectrix show an interference figure consisting of two series of oval-like curves upon which two dark brushes, called isogyres, are superimposed. In the normal position, that is, when the plane including the optic axes and the direction at right angles to it are parallel to the cross hairs (in the ocular), the interference figure resembles Figure 28, 90° position. In white light, the curves are colored, while in monochromatic light they are alternately light and dark. The distance between the optic axes or "eyes" gives some indication of the size of the angle between the optic axes, designated as 2 E. The closer together the "eyes" are, the smaller the angle, and vice versa. (Observe the interference figure given by santonin, C–1, and lactose hydrate, C–16.) The angle of the optic axes (2E) is constant for any given substance and is independent of the thickness of the fragment, provided the temperature remains the same. Figure 28 also shows the biaxial interference figure in the 45° position.

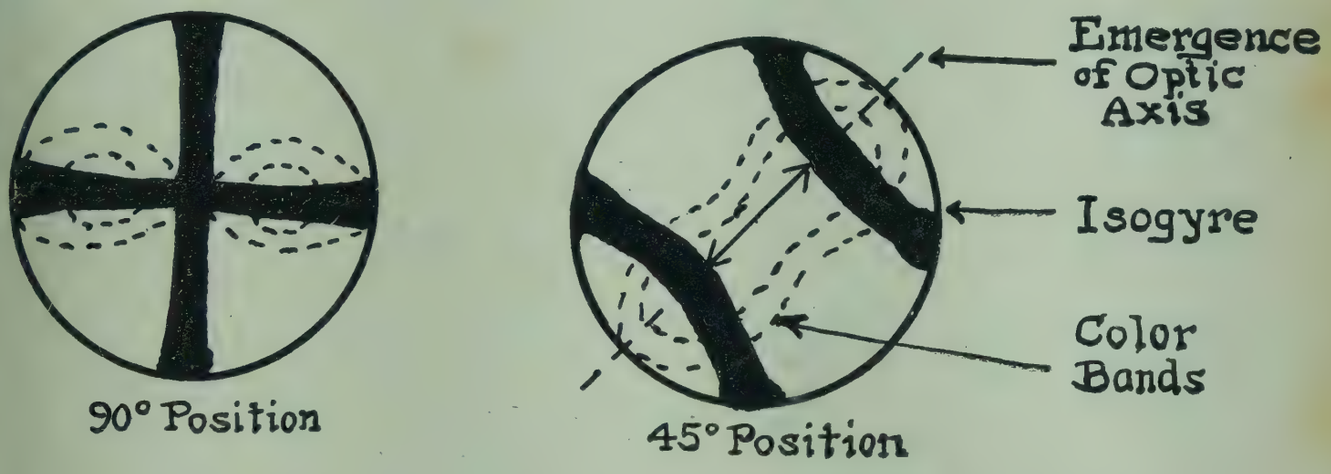

Figure 28.—Biaxial interference figures, 90° and 45° positions.

(b) Crystal Fragments Inclined to the Acute Bisectrix.—Such fragments show a partial biaxial interference figure, usually one optic axis or "eye" and a portion of the brushes being visible. The brushes always move in a direction opposite to that of the stage (Figure 29*A*).

(c) Crystal Fragments Perpendicular to an Optic Axis.—These sections show the emergence of an optic axis, as illustrated in Figure 29*B*. Powdered sucrose when mounted in an oily menstruum (liquid petrolatum is satisfactory) will illustrate this type of biaxial interference.

In contradistinction to uniaxial substances, biaxial crystalline material have three significant refractive indices, frequently designated as n_α, n_β, and n_γ. n_α is the minimum index, n_β the intermediate value, and n_γ the maximum refractive index. From these indices it can be determined whether the biaxial substance is optically positive or negative. If n_α is closest numerically to n_β, then the optical character is positive; if n_β is closest numerically to n_γ the optical character is negative.

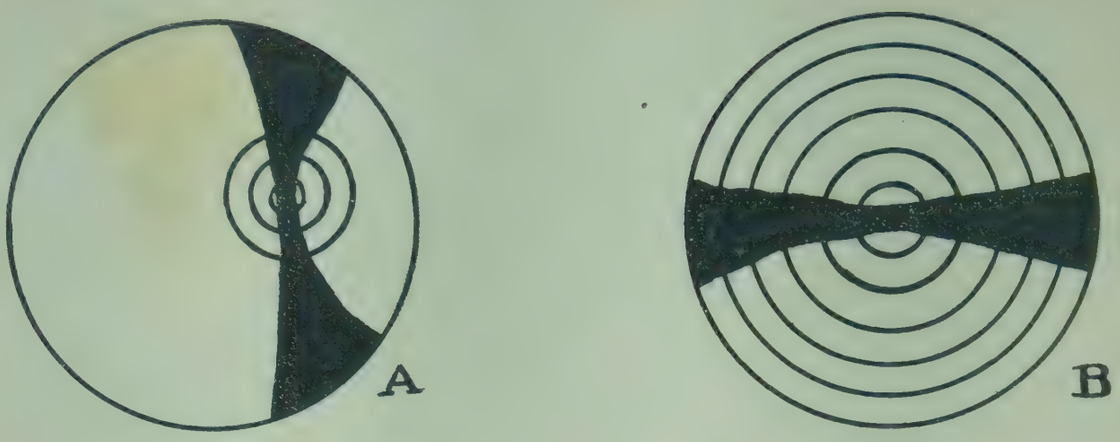

FIGURE 29.—Biaxial interference figures. *A*, Inclined to acute bisectrix; *B*, Perpendicular to optic axis.

E. IMMERSION MEDIA

For the study and examination of crystalline substances with the chemical microscope, it is necessary to mount the material in suitable menstrua. In all microscopic work immersion media of one kind or another are used in preparing mounts for microscopic study. This of course applies to biological material as well as to crystalline substances. Their purpose, primarily, is to aid visibility and, secondarily, in connection with the coverslip, to protect both the object and the objective lens. The preparation of the mount may become a very complicated matter or may be of the utmost simplicity, depending upon the nature of the results to be desired.

The choice of the immersion medium is a matter of the utmost importance, especially in the determination of the refractive indices of crystalline material. For example, if a broken fragment of glass (C–15) is mounted in some oily liquid of known refractive index, different from that of the glass, the glass fragment is easily visible. If, by trial and error, we mount the same fragment of glass in oils of refractive indices progressively nearer that of the glass, the glass progressively becomes less visible and eventually the fragment will become invisible in the proper oil. Thus, knowing the refractive index of the oil in which the glass fragment is invisible, the refractive index of the glass is also determined.

Ideally, immersion media which are to be used for refractive index determinations should have the same color and the same intensity of color as the substance to be examined. They should be stable in contact with air and under the influence of light. Their refractive indices should not vary perceptibly with ordinary changes of temperature and they should not be very volatile. They should not dissolve the substances to be examined, and they should all be mutually miscible so as to enable the worker to form media of refractive indices intermediate between any two given indices. Needless to say, no such ideal set of media has ever yet been contrived. There are sets, however, that are very practicable. Mixtures of mineral oil with $n = 1.490$, monochlornaphthalene with $n = 1.640$, and the methylene iodide with $n = 1.733$–1.740, have been found to be useful.

F. Determination of Refractive Indices by Immersion Method (Becke Line)

The indices of refraction of crystalline solids may be determined by using the immersion method and making observations on the Becke line (Friedrich J. Becke, 1855–1931). This method depends upon refraction and total reflection of light and is known as the method of central illumination.

For the determination of the refractive indices, the crystals or crystal fragments of a given substance are successively suspended in immersion liquids of known refractive indices, advantage being taken of the fact that the greater the difference between the indices of refraction of crystal and liquid, the more prominently the one will stand out in bold relief from the other. By repeatedly mounting such crystals in oils of successively lower or higher index it will be found that ultimately the zone of contact of crystal and liquid becomes practically invisible, therefore demonstrating that the refractive index of liquid and solid has been matched. In the case of substances crystallizing in the isometric (cubic) system, there is only one refractive index, designated by the symbol n. As has already been stated, such substances are not doubly refractive when examined with crossed nicols. Substances crystallizing in other systems, namely, the hexogonal, tetragonal, monoclinic, triclinic, and orthorhombic systems, in the ideal cases, have more than one measureable refractive index. In the case of uniaxial substances such as those crystallizing in the hexagonal and tetragonal systems, two significant indices can be determined, designated as n_ϵ and n_ω. The indices of refraction in the monoclinic, triclinic, and orthorhombic systems, in the ideal cases, are three in number, and designated as n_α, n_β, and n_γ.

The determination of the refractive index of isometric substances is simpler than in the case of doubly refractive material. The substance is successively immersed in liquids of known refractive index until ultimately the zone of contact of crystal with liquid becomes invisible. Then the refractive index of liquid has been matched against that of the solid. Sodium chloride, for example, (C–2) has one refractive index, $n = 1.544$.

The determination of the refractive indices of doubly refractive substances, the uniaxial and biaxial groups, respectively, is somewhat more complicated and will be discussed under those headings.

1. Uniaxial Substances.

The procedure for the determination of n_ϵ and n_ω in uniaxial crystals is as follows. Mount the crystalline material in an oily liquid of known refractive index. To determine n_ω, select a grain which appears black or dark gray during a complete rotation with crossed nicols. Remove the upper nicol and compare the index of the grain with that of the immersion medium. The correct immersion medium will not always be selected the first time. In order to determine whether the crystal fragment or liquid are higher or lower in refractive index, observation is made on the Becke line or the halo of light surrounding the fragment. This halo of light around the fragment is best seen by adjusting the iris diaphragm so that only enough light enters the field for convenient

visibility. The precaution to be observed is to avoid flooding the field with so much light that the Becke line is practically obliterated. A 6x ocular and an 8 mm. objective will in most instances furnish sufficient magnification. Raise the microscope tube slowly by means of the fine adjustment screw. If, when the tube is raised, the band of light (Becke line) moves into the fragment, the fragment has the higher refractive index; if the band of light moves outward (toward the liquid), the immersion liquid has a refractive index higher than that of the crystal fragment. If the index of the liquid is lower than that of the fragment, obviously a liquid of higher value will have to be chosen in order to work in the direction of properly matching crystal against liquid. By this trial and error method eventually an immersion liquid will be found in which the outlines of the crystal fragment have disappeared.

Uniaxial substances with the optic axis vertical (see Figure 28) will give n_ω no matter where the stage is rotated. Uniaxial substances with the optic axis horizontal give n_ω at one extinction position and n_ϵ when rotated 90° to the next position of extinction (Figure 6a). Every orientation, therefore, permits the measurement of n_ω, but only one orientation allows the measurement of n_ϵ. Frequently it will not be possible to obtain interference figures on the substance. In that case, if the refractive indices are determined for two positions of extinction of a number of fragments in random orientation, it will be found that one of the two values will be the same in all the grains. This constant index is n_ω. The other value will vary from grain to grain, depending upon the orientation. Statistically, the highest and lowest indices determined on a large number of grains of the same substance in random orientation will be n_ω and n_ϵ, or n_ϵ and n_ω, respectively, depending upon the optical sign of the substance (discussed subsequently under "Microscope Accessories").

2. Biaxial Substances.

This group will be more frequently encountered than the uniaxial substances. If interference figures can be obtained on the substance (convergent polarized light, crossed nicols), a fragment is selected which gives an interference figure showing the acute bisectrix (Figure 28*B*). Two indices of refraction can be determined on such a fragment, one in one extinction position, and the other 90° removed from the first. One of these two indices will be n_β and the other may be n_α or n_γ depending upon whether the grain is optically positive or negative (discussed subsequently). If the grain is positive, the lower of the two indices is n_α and the higher n_β. If the grain is optically negative, the higher of the two indices is n_γ and the lower n_β. Selecting grains that do not extinguish sharply with crossed nicols may show an interference figure perpendicular to an optic axis (Figure 8), in which case n_β can always be measured. If the crystalline material does not show interference figures, which frequently is the case with organic substances, the statistical method has to be resorted to. Measure the lowest refractive index in any given mount. This will be n_α. Then measure the highest shown by any grain. This will be n_γ. Usually two such indices will suffice to characterize the substance, in view of lack of interference figures.

Lactose hydrate (C–16) illustrates a biaxial substance with significant microscopic-crystallographic properties that are useful in its identification. Biaxial interference figures showing the acute bisectrix are common and the optic sign is negative. Those fragments, therefore, showing such a figure and with negative optic sign will give $n_\beta = 1.542$ in one extinction position and $n_\gamma = 1.550$ in an extinction position 90° removed from the first. The lowest refractive index measureable on the substance is $n_\alpha = 1.517$.

G. Microscope Accessories

There are a few accessories frequently provided with the chemical microscope known as compensators. The most important ones are the quartz wedge, gypsum plate (also called "1st order red," "selenite," and "unit retardation" plate). The two compensators most frequently used by the analyst will be the quartz wedge and the gypsum plate.

1. Quartz Wedge.

This compensator consists of a wedge of quartz so ground as to produce interference colors ranging from the first to the third or fourth order of the spectrum. There is a slot provided in the microscope tube for the insertion of this wedge (Figure 21). When this plate is pushed into the slot, with its thin edge foremost, it will show parallel bands, varying from nearly darkness to colors of the higher orders. This can be explained by the fact that as the wedge is moved into the field, its increased thickness causes more and more retardation of the transmitted rays. The observation of a crystal fragment when this wedge is used will demonstrate a gradual reduction of the interference colors to zero when the vibration directions of the two are at right angles. When the retardations of the two are equal, a black band will appear. This is termed the POINT OF COMPENSATION. In determinative work the quartz wedge will not find as wide application as the gypsum plate.

2. Gypsum Plate.

The positive or negative character of both uniaxial and biaxial crystals can be determined by this plate (Figure 30). Like the quartz wedge it is inserted in the slot of the microscope tube and used in conjunction with crossed nicols and the interference figure (convergent polarized light).

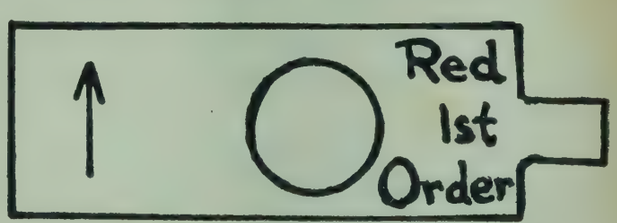

FIGURE 30.—Unit retardation plate.

Figure 31 illustrates the effect of the unit retardation plate on positive and negative uniaxial interference figures. In the quadrants of the interference figure in which the relative retardation is increased, the margin of the brushes (isogyres) will be blue while the opposite quadrants will be yellow. The disposition of these colors for the positive and negative crystals are illustrated below.

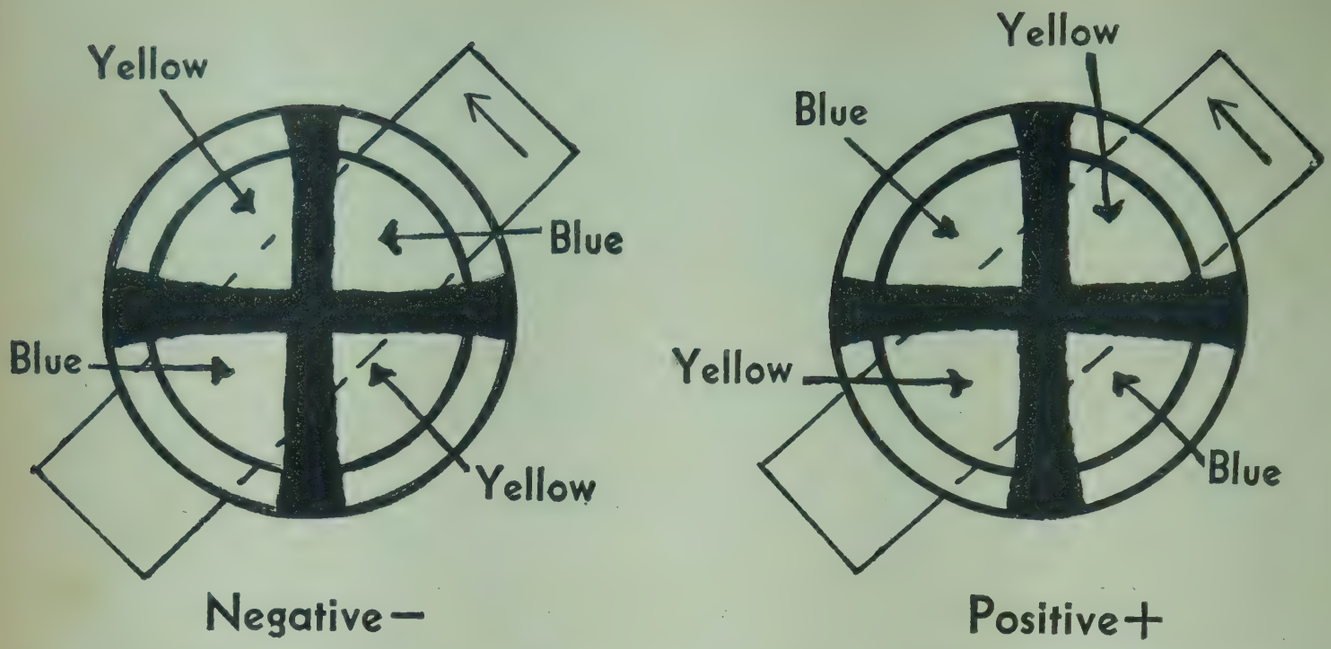

FIGURE 31.—Effect of unit retardation plate on positive and negative uniaxial interference figures.

The phenomena exhibited for biaxial interference figures are somewhat analogous, although as indicated previously, the nature of the interference necessarily is different, resulting in a rearrangement of the yellow and blue areas as distinguishing a positive crystal from a negative one. Figure 32 shows the disposition of the colored fringes for positive and negative biaxial interference figures.

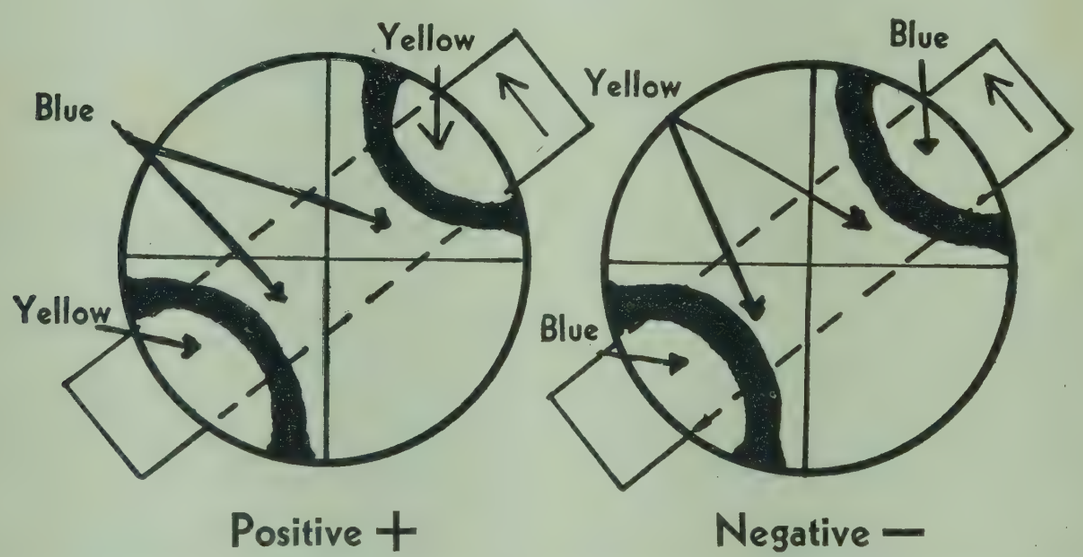

FIGURE 32.—Effect of unit retardation plate on positive and negative biaxial interference figures. (Acute bisectrix.)

The sign of elongation (positive or negative) is also determined with the gypsum plate and crossed nicols. A long and narrow crystal, showing very little color with crossed nicols, is so oriented that its long dimension is parallel to direction "c" of the plate which is inserted in the slit of the microscope tube. (Direction "c" is indicated by an arrow, as shown in Figure 32.) If the crystal appears blue or other color of a higher order than the red-violet due to the plate, the elongation is positive; if the crystal appears yellow, white, or gray, that is, of lower order color than the red-violet field, the elongation is negative.

H. Description of Slides of Authentic Materials

The following examples have been chosen to illustrate principally the different types of habit found in some common chemical compounds. In the preparation of these permanent mounts of crystalline material, it is apparent that few characteristics other than the habit and reaction to parallel polarized light (crossed nicols) can be shown. Temporary mounts prepared from authentic specimens will furnish additional data illustrative of the preceding descriptions.

C–1. Santonin.—Santonin is the anhydride of santoninic acid and is the well-known anthelmintic used to expel roundworms and threadworms. It crystallizes in the orthorhombic system and therefore is doubly refracting. When examined with convergent polarized light (crossed nicols), acute bisectrix figures are common, and show that the optic sign is positive.

C–2. Sodium Chloride.—This is a common example of a substance crystallizing in the isometric (cubic) system. There are a number of other salts frequently encountered in the laboratory which are also isotropic like sodium chloride, crystallizing in the isometric system. Therefore, these microscopic-crystallographic features are not specific for sodium chloride but rather the refractive index which is 1.544.

C–3. Ephedrine Hydrochloride.—This is the readily soluble salt of the alkaloid employed in many preparations for the relief of congestion due to common colds. It occurs as well-developed elongated crystals, terminated at both ends, and six-sided plates. The polarization colors are brilliant when examination is made of the material with crossed nicols, and many of the crystals do not extinguish sharply under these conditions when the microscope stage is revolved. The following refractive indices have been recorded for this substance: $n_\alpha = 1.530$, $n_\beta = 1.603$, $n_\gamma = 1.638$.

C–4. Quinacrine Hydrochloride.—The well-known synthetic antimalarial, also known as atabrine or atabrin, crystallizes in yellow rhombs or parallelograms which extinguish sharply with crossed nicols. Frequently the commercial samples can be recrystallized from 95% alcohol to show the characteristic rhombic plates. The two most significant refractive indices for the substance are the following: $n_\alpha = 1.522$, and an intermediate value which agrees with that for methylene iodide, 1.733. The maximum refractive index is higher than 1.733.

C–5. Cocaine Hydrochloride.—This substance as usually found in commerce consists of very thin, micaceous plates which extinguish sharply with crossed nicols and do not exhibit any brilliant polarization colors. The substance crystallizes in the orthorhombic system with the following significant refractive indices: $n_\alpha = 1.570$, $n_\beta = 1.596$, $n_\gamma = 1.618$.

C–6. Dextrose Hydrate.—Known commercially as corn sugar, this usually consists of plates of hexagonal outline, and crystallizes in the monoclinic system with the following two refractive indices as significant for the substance: $n_\alpha = 1.521$, $n_\gamma = 1.549$.

C–7. Pumice.—A volcanic glass, pumice is sometimes used as an inert filler or carrier in some types of mixtures used for dusting purposes. It resembles other glasses in that it is amorphous and therefore does not exhibit any double refraction with crossed nicols.

C–8. Sulfathiazole.—One of the common "sulfa" drugs, sulfathiazole is frequently found in commerce in the form of small elongated prisms and plates which are doubly refracting, exhibiting brilliant polarization colors with crossed nicols. Under certain conditions of crystallization, sulfathiazole is dimorphic. The significant refractive indices of the commonly occurring form are: $n_\alpha = 1.674$, $n_\beta = 1.685$, $n_\gamma = >1.733$.

C–9. Codeine Sulfate.—This substance is frequently found in cough remedies. It crystallizes in the orthorhombic system, usually taking the form of broad rods showing parallel extinction and brilliant polarization colors with crossed nicols. The following refractive indices are significant for the substance: $n_\alpha = 1.561$, $n_\beta = 1.642$, $n_\gamma = 1.661$.

C–10. Sulfaguanidine.—This substance crystallizes in rather massive, elongated prisms and irregular forms, which are doubly refractive but examination with crossed nicols shows that the extinction is rather weak.

C–11. Quinidine Sulfate.—Another example of the cinchona alkaloid group, quinidine sulfate, like the other alkaloids of cinchona bark, has been employed as an antimalarial and in tonics. It crystallizes in broad rods showing parallel extinction and brilliant polarization colors when examined with crossed nicols. The following refractive indices are significant for the substance: $n_\alpha = 1.565$, $n_\beta = 1.607$, $n_\gamma = 1.670$.

C–12. Sodium Bicarbonate.—This common substance crystallizes in the monoclinic system, and therefore is doubly refractive. Its habit is quite characteristic and useful in identifying the substance objectively in mixtures. The frequent occurrence of twins and "elbow" forms is a valuable clue as to the presence of the substance. The significant refractive indices are: $n_\alpha = 1.380$, $n_\beta = 1.500$, $n_\gamma = 1.586$.

C–13. Calomel.—Mercurous chloride is doubly refractive with very high refractive indices, and therefore not practicable for measurement by the immersion method. The substance frequently occurs, even in tablet mixtures, in the form of compact, small, rosette aggregates of prisms.

C–14. Quinine Sulfate.—This salt of the common cinchona alkaloid is usually found crystallized in rods and needles. The rods often have a checked appearance, probably resulting from the loss of some water of crystallization. The following refractive indices have been found to be significant for the substance: $n_\alpha = 1.595$, $n_\beta = 1.635$, $n_\gamma = 1.690$.

C–15. Glass.—Powdered glass is amorphous, consisting of irregular fragments not exhibiting double refraction with crossed nicols. The refractive index of glass varies with its composition.

C–16. Lactose Hydrate.—Lactose or milk sugar is frequently found as a diluent in drug preparations, especially tablets and powders in capsules. It crystallizes in the monoclinic system with one molecule of water of crystallization. It is especially characterized by its habit, most frequently being wedge-shaped forms or darts. Even in powdered mixtures, such as tablets, this habit is readily observed. The following refractive indices are significant for the substance: $n_\alpha = 1.517$, $n_\beta = 1.542$, $n_\gamma = 1.550$.

C–17. Cinchonidine Sulfate.—This alkaloidal salt crystallizes in rods which are doubly refracting, showing brilliant polarization colors with crossed nicols, and parallel extinction.

C–18. Morphine Hydrochloride.—The hydrochloride of this opium alkaloid crystallizes in rods which are doubly refractive and show parallel extinction with crossed nicols. The following refractive indices have been found significant for the substance: $n_\alpha = 1.540$, $n_\beta = 1.590$, $n_\gamma = 1.635$.

C–19. Talc.—This hydrated silicate of magnesium crystallizes in very thin, plate-like fragments which have a tendency to rest edgewise in the oily menstruum, suggesting the appearance of needles in this orientation. The substance is doubly refractive, exhibiting low order interference colors with crossed nicols, and crystallizes in the monoclinic system. The following refractive indices are significant for the substance: $n_\alpha = 1.539$, $n_\beta = 1.589$, $n_\gamma = 1.589$.

C–20. Orris Root.—This slide is included to illustrate the elongated prisms of calcium oxalate monohydrate, a common crystalline ingredient in many plant materials. These elongated prisms in orris root are best observed in parallel polarized light (crossed nicols). Calcium oxalate monohydrate crystallizes in the monoclinic system with the following significant refractive indices: $n_\alpha = 1.490$, $n_\beta = 1.555$, $n_\gamma = 1.650$.

C–21. Rhubarb Rhizome and Root.—This material illustrates the large rosette aggregates of calcium oxalate which are very numerous in rhubarb and especially observed with crossed nicols.

C–22. Squill.—Powdered squill bulb illustrates the needle-shape form of calcium oxalate, occurring in short and long bundles. The needles are frequently referred to as raphides. These are best observed with crossed nicols.

C–23. Arsenic Trioxide.—White arsenic, as it is frequently known, crystallizes in distinct octahedra (isometric system) and therefore does not exhibit double refraction with crossed nicols. Its significant refractive index is 1.755.

C–24. Boric Acid.—Boric acid crystallizes in the triclinic system with three molecules of water of crystallization. The substance is therefore doubly refracting, invariably occurring in fragments showing a cross-hatched or fibrous appearance, especially noticeable with crossed nicols. The significant refractive indices are: $n_\alpha = 1.340$, $n_\beta = 1.456$, $n_\gamma = 1.459$.

C–25. Sodium Benzoate.—This substance crystallizes in very fine needles which are doubly refractive. The following refractive indices have been found to be significant for the substance: $n_\alpha = 1.490$ and $n_\gamma = 1.680$.

C–26. Sodium Salicylate.—The habit of this substance is not significant; it invariably occurs in irregular fragments which are doubly refractive. The following refractive indices have been found to be significant: $n_\alpha = 1.490$ and $n_\gamma = 1.680$.

C–27. Sodium Glutamate.—This is also known as mono-sodium glutamate and is frequently used to impart a meat flavor to foods, especially soups. The substance crystallizes in rods which are doubly

refractive and show weak extinction with crossed nicols. The following refractive indices have been found to be significant: $n_\alpha = 1.495$ and $n_\gamma = 1.593$.

C–28. Silica.—Silica exists in several crystal phases but the low temperature phase is the very common substance known as quartz (SiO_2). This crystallizes in the hexagonal system and therefore, in contradistinction to glasses, is doubly refractive when examined with crossed nicols. The habit of powdered quartz is not significant. The following refractive indices are significant for quartz: $n_\omega = 1.544$ and $n_\epsilon = 1.553$.

VII. HISTOLOGY OF FOOD AND DRUG MATERIALS

A. General Discussion

These methods involve the use of biological microscopes as employed in the study of plants and animals from the standpoint of morphology and histology. They assume a familiarity on the part of the worker of the basic principles of animal and vegetable morphology and histology. Animal and vegetable food and drug products usually consist of some special part of the plant or animal, but occasionally the entire plant or animal may be used. We know that different species of organisms differ in both their gross morphological and finer cellular structures. Different organs and tissues from the same species will differ similarly. Thus it becomes possible to identify animal and vegetable products present as such in foods and drugs largely from their diagnostic morphological and cellular elements. Most materials will be in a comminuted form when submitted for analysis, compelling the analyst to note cellular details revealed only under high magnification.

Generally, degrees of relationship will be indicated by certain similarities of diagnostic elements enabling the grouping of substances into a classification scheme. Thus, leaves will be indicated by the presence of an epidermis with stomata and hairs, palisade tissue, an abundance of chlorophyll, and veins and veinlets; barks by the presence of abundant bast, corky tissue, and calcium oxalate crystal fibers; seeds by the presence of aleurone grains, reserve starch, fixed oil, or fat; animal meat meals by the presence of muscle tissue, etc. Such a grouping is based on the parts of the plant or animal used. Another system of arranging these substances is according to their important constituents, such as starch grains, oil seeds, alkaloid materials, etc. A third method is to arrange plant and animal materials according to their natural relationship following the scheme of the systematic taxonomist of grouping organisms into orders, families, genera, and species. Still other arrangements may be based on the specificity of starch grains or pollen grains in plant substances or hairs in animals.

Whatever classification system or scheme is used, all depends upon structural similarities and dissimilarities between organisms and their parts as revealed to the critical observer. These systems present the analyst with diagnostic elements which he can utilize in solving his particular problems of identification. It is not the purpose of this discussion to go into the relative virtues of one method of classification over another. The diversity of the subject makes it impracticable to adhere to any single system. The analyst cannot always foresee what diagnostic criteria a particular sample will reveal. He must be in a position to utilize his background of experience gained from a study of the structural morphology and histology of plant and animal materials, and may devise his own system of classification to solve a particular problem. Some materials are quite readily distinguished under the microscope, i.e., the polygonal starch grains of corn from the lenticular ones of wheat. Others will call upon the worker's utmost ingenuity, requiring him to pay particular attention to minute differences between closely related substances, as in differentiating chicory and dandelion roots. Here the microscopic structure of the two roots is quite similar. However, the greater length of the pores in the vessels of the dandelion root serves to distinguish it from chicory.

B. Description of Slides of Authentic Materials

1. Wheat, Rye, and Barley Tissues.

Wheat, rye, and barley tissues resemble each other histologically, although they do have some tissue elements, together with the starch, that are valuable in differentiating them. Therefore, for illustrative purposes the most diagnostic tissue for each of the cereals has been selected. The permanent mounts, in many instances not ideal, can be supplemented by the preparation of temporary slides from authentic samples of the ground cereals. Such samples ground from the whole grain furnish an abundance of sections of the fruit coat (bran) in various orientations and are the most suitable and practical for microscopic study. The microanalyst will find in such preparations the commonly occurring sections usually encountered in commercial mixtures of these cereals. It is therefore emphasized that such temporary mounts be used, also free-hand sections of the grain to show the relationship of the various tissue systems. For the examination of the starch, distilled water is the suitable menstruum. The study of the tissues is best carried out if the material is warmed in a small amount of dilute chloral hydrate solution. This procedure serves to dissolve the starch and other chloral hydrate-soluble materials, thus "clearing" the preparation and causing the tissues to stand out more distinctly.

C–37. Wheat Tissue.—Among the most significant tissues of wheat bran, invariably found as fragments in highly refined patent flours, are the cross cells shown in surface view. These cells, as in rye, cross those of the outer layers at right angles and are characterized by numerous distinct pores in the walls, but lack the swollen or thickened end walls as in rye. From a diagnostic standpoint, these cells in surface view are the most important of the wheat bran tissues.

C–38. Rye Tissues.—As in wheat, the cross cells, here illustrated, cross those of the outer layers at right angles, being arranged side by side in rows, and do not form "break joints" as in the case of the wheat cross cells. The walls are not as distinctly porous as in wheat, and, in addition, the end walls are often rounded and thickened. These cells in surface view, together with the starch, are important diagnostic characters for rye.

C–39. Barley Tissue.—The whole barley grain is used for human food only in the roasted form as a coffee substitute. The roasting, however, usually leaves the grain intact enough for identification as barley. Also, the histological elements are sufficiently preserved for microscopic identification. Barley starch closely resembles wheat and rye, consisting of both large and small grains, although the barley grains are generally smaller. In cross sections the endosperm differs from that of all other cereals in that it is two to four cell-rows thick. In comminuted or milled products, cross-sections of the grain are not frequently encountered. The most significant tissue element frequently found in ground barley products is that showing the two layers of thin-walled cross cells (as illustrated in slide). As in the case of the other cereals, temporary mounts cleared in diluted chloral hydrate solution can be readily prepared from ground barley and are much more satisfactory than permanent mounts. The starch, of course, is best studied in a water mount.

2. Common Commercial Starches.

Numerous foods contain starch but only a comparatively few are suited for the manufacture of starch for commercial purposes. The raw material available in each region, the quantity of yield, also the quality of the starch, determined by its physical properties, will determine its suitability for commercial use. On the European Continent potato and wheat are the most important starches. In England, rice starch is more commonly used, while in the United States, corn starch is most widely distributed. Other starches are of interest primarily in connection with their histological characters that are descriptive of the particular plant material.

Nägeli (1858) as a result of his classic work on the starch grain advanced the theory that the grain is composed of ultramicroscopic crystalline particles which he called "micellae," these being surrounded by water films of varying thickness. It was similarly held by A. Meyer (1883-1885) that the starch grain is composed of radially arranged needle-shaped crystals known as "trichites." Nägeli and Meyer both attributed the stratification of the grain to the varying numbers of the crystalline units in the successive layers.

The behavior of starch grains toward polarized light seems to have been discovered by Biot (Compt. rend. 1844, XVIII, 795). Since then many observers have noted the differences in the form and distinctness of the interference figure or "cross." The point of intersection of the two parts of the cross usually corresponds to the position of the hilum. This point is the organic center about which the starch grain is formed in successive layers. This point may be centric or eccentric, distinctly visible in some varieties of starch.

The refractive indices of various starches have been determined but these are not of significance from a determinative standpoint.

The main constituents of ordinary starch are d-amylose and b-amylose, the latter turning a blue color with iodine solution. Other starches in which the dextrin constituents predominate turn brown or reddish with iodine solution.

The following examples have been chosen to illustrate different types of starches encountered in microanalysis:

C–29. Corn Starch.—The large grains are for the most part sharply polygonal, much less often round, measuring up to 30 microns in diameter. The hilum is centric and very distinct. When examined with crossed nicols, the polarization cross is very distinct.

C–30. Potato Starch.—In some samples of the starch, the grains are large enough to be visible to the naked eye. The large grains are ellipsoidal, ovoid, or irregularly lobed, truncated, and varying in length up to 100 microns. The hilum is distinct, usually in narrow end of grain, sometimes double. Rings, which are eccentric, are distinctly shown when the microscopic field is not too bright. The polarization cross is very distinct and eccentric.

C–31. Rice Starch.—This is one of our smallest commercial starches and might be compared with oat starch. The grains are mostly polygonal with a diameter from 2 to 10 microns. The grains are present in the kernel frequently in aggregates but these are largely disintegrated in the process of manufacture. The hilum is centric and small, and the polarization cross distinct.

C–32. Oat Starch.—This starch is not of commercial importance but is presented for comparison with rice starch, with which it might be confused. Like rice starch, oat starch has small polygonal grains measuring up to 10 microns in diameter, these frequently being united in aggregates. Spindle-shaped grains and the occurrence of the aggregates serve to differentiate it from rice starch.

C–33. Wheat Starch.—The larger wheat starch grains are lenticular in shape (elliptical when turned on edge), the diameter commonly being 28 to 40 microns, rarely 50 microns. The hilum is centric and the polarization cross often is indistinct.

C–34. Rye Starch.—This is not a commercial starch but is of interest in connection with the examination of milled rye products. The starch grains are very similar to those of wheat, although their average and maximum size is somewhat larger. There are apparently more distinctly round grains in rye starch than in wheat. The presence of slits or radiating fissures in rye starch grains have sometimes been regarded as diagnostic although this character is not always reliable.

C–35. Pea Starch.—This starch represents the leguminous type. The grains are oval-oblong, rounded, or sub-reniform in shape. The appearance of the hilum varies, in some grains being a conspicuous dark central cleft, but frequently not as distinct as the hilum of bean starch grains. The polarization cross is not as distinct as in corn and potato starches, but nevertheless is characteristic of the legume type.

C–36. Cassava Starch.—Cassava starch is produced from two important food plants, the bitter cassava, *Manihot utilissima Pohl,* and the sweet cassava, *M. aipi Pohl.* The large grains are mostly kettle-drum shaped, or truncated, and have a diameter up to 35 microns. The hilum is usually centric and distinct. The polarization cross is very distinct.

3. Berry Seeds and Apple Skin.

Several authentic slides have been made of various types of fruit seeds. These are of value in the identification of the fruit itself.

A–1. Cuthbert Red Raspberry Seeds are usually small in size, approximately 2.3 mm. long by 1.3 mm. wide. They are a light yellow color.

A–2. The seeds of the Columbian Red Raspberry have a slight purplish tinge and are slightly larger than the Cuthbert red raspberry seed, being approximately 2.4 mm. long by 1.4 mm. wide.

A–3. Seeds of Black Raspberry have a slightly darker purplish color and the pit depressions are about the same as in the red raspberry seeds. They have an average size of approximately 2.4 mm. long by 1.3 mm. wide.

A–4. Seeds of Blackberry differ from the raspberry seeds in that they have more pronounced pits, are dark purplish color and slightly larger in size, being approximately 3.1 mm. long by 2.0 mm. wide.

A–5. Seeds of the Loganberry are a light pink color and are long and narrow, measuring approximately 3.0 mm. long by 1.5 mm. wide.

A–6. Boysenberry Seeds are rather large in comparison with other berry seeds, measuring approximately 3.7 mm. long by 2.1 mm. wide. They are light-colored, being somewhat similar to loganberry seeds.

A–7. Strawberry Seeds are small and of a light yellowish color. The seeds are somewhat triangular in shape.

A–8. Fig Seeds are somewhat similar to strawberry seeds in color but differ in size and shape. The seeds are smaller and more oval than strawberry seeds.

A–9. Apple Skin Tissue is characterized by the window-like grouping of cells.

VIII. PHOTOGRAPHY

A. Use of the Camera

The camera is proving to be a valuable adjunct to the study of extraneous matter found in foods and the preparation of exhibits. These may be used to train analysts or to present to the court in the event of a trial. Some of the most variable and inconsistent results are obtained by an operator who takes pictures as directed by some little-understood set of directions. It is really simpler in the long run to obtain good photographs by understanding a few general principles and memorizing some few data such as the characteristics of the particular film and flash bulb.

A camera consists of a lens, shutter, and film-carrying device in a light-tight box. Films or photographic plates vary in their sensitivity to light as a whole and to various colors of the spectrum. Some are extremely sensitive to all visible colors (Eastman Super XX, Agfa Ultra Speed). Another group is sensitive to all visible colors but less rapid in their reaction to light (Eastman Plus X, Agfa Superpan Supreme). These photographic emulsions, sensitive to all colors to approximately the same degree as is the human eye, are said to be "panchromatic." Another group (including Eastman Verichrome, Agfa Plenachrome) are said to be "orthochromatic" and are relatively insensitive to red, the red colors of the object registering as black on the photographic emulsion. Each type of photographic plate has its own advantages and within each group there are additional types with a variety of speeds, graininess, contrast, developing times, etc., to choose from. It should be remembered that a "fast" film is one that is more senstive to light and not one that develops rapidly. For general Food and Drug use it is well to learn the characteristics of one film (e.g., Eastman Plus X or Agfa Superpan Supreme) of the so-called general all-purpose type and use it repeatedly before trying others.

Almost limitless types of developers can be purchased or mixed in the laboratory. Again each has a certain quality which the others do not have which "brings out" certain desirable features from the film. Some are more "contrasty," some less; some fast, some slow; some can be used repeatedly, some a few times; some are secret formulas, while some are well known. Certain general purpose fairly contrasty developers, such as Eastman D-76 or Agfa 17, can be used in the beginning and in fact they may be used even for advanced types of work. With most packages of films or printing papers a developer is suggested and that one invariably proves useful. Each film is developed for a certain time and at a certain temperature (the panchromatic films ordinarily cannot be examined while developing), then fixed, washed, and dried to remove the undeveloped chemicals and preserve the emulsion.

It is necessary to expose the film to the correct amount of light. Moreover, the light must be focused on the film. To do this, the light-tight box must have a lens and a shutter. The amount of light falling on the sensitized film may be varied in three ways. (1) The subject may be illuminated more or less intensely. (2) The lens opening may be made larger or smaller to permit a larger or smaller cone of light to enter. (3) The hole in the camera through which the light enters

may be opened for a longer or shorter interval of time. The ordinary box camera has a fixed shutter speed and fixed lens size (diaphragm opening). Hence with it, photographs must be taken within a small range of illumination which for cheapness of manufacture is fixed at bright sunlight. In subdued light the amount of light entering is not enough to cause a reaction in the film.

Photoflash or photoflood bulbs can be used to illuminate the object if the normal illumination is not sufficiently bright. For use during inspections the flash bulbs, which require no electrical connections and interfere less with plant operations, are to be preferred to photofloods. When the flash unit is attached to the camera there is no problem of pointing the camera and the illuminant for they move together. It is necessary only to follow the directions which the manufacturer supplies for his bulbs and exposure will be correct. Films have a certain amount of latitude and the directions may be varied somewhat without markedly influencing the results, although it must be remembered that illumination varies with the square of the distance. In using a photoflood or photoflash bulb to supplement ordinary indoor lighting, practically no allowance need be made for the amount of light contributed by the normal illuminants. While manufacturers' charts on the use of photflash bulbs give directions for the use of the bulbs with cameras equipped with focal plane shutters, the use of such shutters necessitates the use of special bulbs with a relatively long period of maximum illumination so that the entire film surface is evenly illuminated. Thus the use of a focal plane shutter introduces difficulties of exposure which are not present with between-the-lens shutters. Such cameras must either be used with shutter speeds of $\frac{1}{100}$ second or faster or with the shutter set at bulb or time and with the flash bulb giving the period of illumination.

In addition to varying the intensity of illumination on the subject, the lens opening or "f" number may be changed.[1] This number represents the so-called "speed" of the lens. The smaller the "f" number the faster the lens and, other factors remaining constant, as the illumination decreases it is possible to compensate for this decrease by opening the lens diaphragm to a lower "f" number. This adjustment has several limitations relating to general optical theory which will be discussed here only by mentioning one point, depth of focus.

Obviously, the camera must be "focused" so that the object being photographed is projected sharply upon the negative. To understand this feature it is necessary to return to the concept of "f" number or aperture (Figure 33).

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[1] The lens "f" number is simply a convenient way of expressing the amount of light-gathering power of a lens. This power is dependent upon the relationship of two factors: The diameter of the film and the diameter of the lens. Obviously, the greater the diameter of the lens in proportion to the film size, the greater will be the light-gathering power of the lens. In practice, the distance from the lens to the film (focal length) is used in place of the film diameter. The ratio of focal length to lens diameter is known as focal ratio. If a lens has a focal length 4.5 times as large as the lens diameter it has a focal ratio of 4.5. The "f" number is the reciprocal of the focal ratio. Thus for the lens postulated above, the "f" number would be simply f/4.5, the lens diameter being 1/4.5 of the focal length.

Practically all photographic objectives are composed of more than one glass element but if they are considered as one unit (L) an image of a point of light being formed on film (F) by rays AF and BF will be in focus only on one relatively thin plane. However, if a small

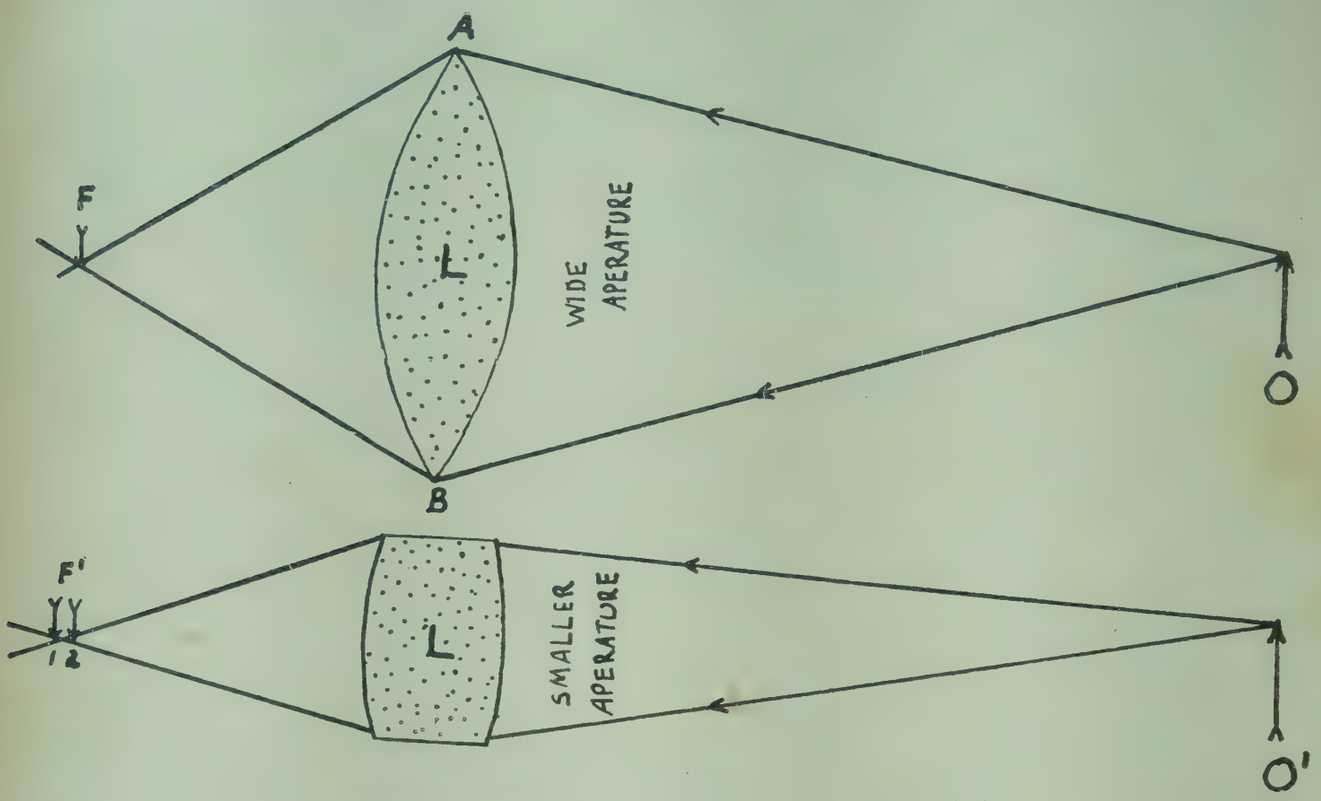


FIGURE 33.—Aperture in relation to depth of focus.

aperture is used then a clear image is formed along a relatively thick "plane" and for all practical purposes the eye could not distinguish between the image formed at F1 or 2. In actual practice, the film is placed in a certain plane for maximum resolution and the objective is focused on some point which is projected on the film. Now it is seen that just as there is more depth of focus on the film when a small aperture is used, so there is more depth of focus on the object (O) when a small lens aperture (high "f" number) is used. With a large lens opening only the object O would be sharply imaged and objects closer or further away would be out of focus, but with a small lens opening, O′ and objects closely surrounding it would, for all practical purposes, be in focus.

One practical limit to film size is that as film size increases the depth of focus, compared with an equal "f" number on a smaller film, falls off. At any rate, the camera should be focused accurately and the larger the film used, or wider the aperture, the more exact this focusing must be, and similarly the less will be in focus at one time. Usually there is more in focus behind the subject being photographed than there is in front of it.

Finally, the shutter may be left open for a longer or shorter period of time to increase or decrease the amount of light falling on the sensitized film. The use of long time exposures is limited to conditions in which the camera and subject can be held stationary. When there is movement in the subject, speeds of $\frac{1}{100}$ second or shorter should be used. One-twentieth of a second is about the longest exposure that can be given with a manually held camera.

Neither large lens openings nor prolonged exposures will give a negative showing essential details if there is not sufficient illumination to provide contrasting lights and shadows. In a dimly lighted warehouse corner an observer can move from place to place building up an impression of depth, dirt, etc., but a photograph must "look" from one position, and to do this the subject must be well-illuminated. Contrast may be improved upon during the film processing and printing but once the exposure is made such manipulations are sharply limited.

In the inspection of a factory or warehouse it is often desirable to obtain, for possible future court action, photographs of rodent excreta. Most inspectors have had the experience of attempting to photograph such filth near or on some food machinery, and have found after development of their negatives that what they actually saw as fresh, repugnant masses of excreta, in some cases with the actual rodent hairs visibly attached to them, turned out to be a few nondescript spots on the film. It is practically impossible to demonstrate this type of filth with the ordinary camera where it is necessary to take the picture at distances of 3 feet or more. However, it is possible to reproduce the actual picture the inspector sees by proper lens attachments on the ordinary camera. For instance, by the use of a portrait or similar lens it is possible to obtain a sharp focus at distances of as little as 6 inches. In some cameras this also may be accomplished by a bellows attachment. Photographs taken by the use of such accessories will make it possible for the inspector to bring to the court a picture of the filth that he saw in the plant which is nearly as impressive to the court as it was to the inspector when he observed it.

B. Photographic Microscopy

Closely allied to both microscopy and photography is photomicrography. However, the photography is very elementary and the difficulties are encountered in the microscope setup. Once the microscope image is obtained the only thing that remains to be done is to put a plate in the camera in place of the ground glass and expose and develop it.

When making visual observations, either microscopic or otherwise, the eye compensates for certain aberrations which may be present. It can change both its focus and field of view so that the mind builds up a composite picture from a number of impressions. This is not possible on one photographic negative. If the depth of focus of the objective limits the field to one surface of an insect then that is all that goes in one picture and we cannot reproduce in the negative the same impression of depth that is obtained when we visually examine that insect by continuously changing the focus. We can introduce lenses that give increased depth of focus but then either the magnification or resolution will be diminished. With these limitations in mind we can outline briefly certain fundamental steps in photographic microscopy.

Mounting material is no different than that discussed earlier for the preparation of material for microscopic observation. One point, however, has added significance: The mounting medium should be sufficiently stable that it will not evaporate, or flow, or form bubbles to any significant extent while the exposure is being made.

Microscope equipment is similar to that used for visual work, but in addition some sort of a bellows and plate holder arrangement is needed. Visually the eye picks up a VIRTUAL image which is in turn re-focused by the eye lens so that a REAL image is projected onto the retina. The function of the bellows (some cameras have metallic arrangements by which the bellows effect is obtained) is to permit the placing of a photographic plate at the plane of a real image projected by the microscope eyepiece. Many simple gadgets have been devised to effect this bellows extension by simply clamping a bellows and plate holder onto a microscope, fastening an ordinary landscape camera lens onto a microscope, etc. All such temporary equipment has one common disadvantage—vibrations and minor movements ruin the definition especially when a shutter mechanism is used attached to either microscope or camera.

If a photomicrographic setup is contemplated, a choice must be made between rapidity of operation and resolution. Pictures satisfactory for exhibit purposes or court presentation can be made using an ordinary roll-film camera with microscope adapter. It is necessary to focus either on a ground glass or by some empirical rule. The longer the film roll the more pictures can be taken with one sitting or development time. For this type of work 35 mm. film is easily handled both for exposure of the negative and the printing of a long series of pictures.

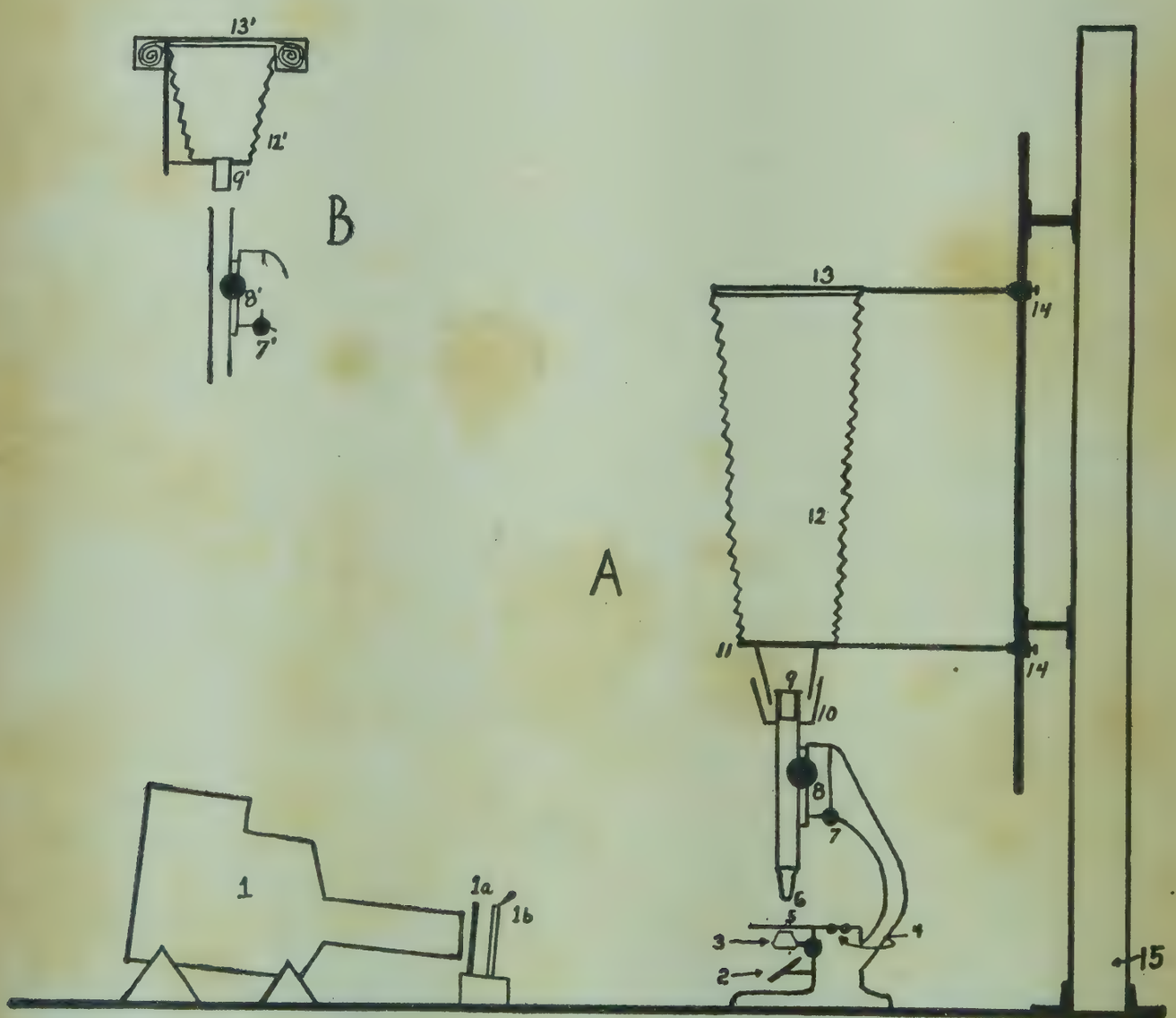


FIGURE 34.—Arrangement of equipment for photomicroscopy.

Pictures for publication or for reference usually are made with heavier equipment and with a single plate for each negative.

The simplest way to discuss these features is by reference to the figures depicting the arrangement of equipment for photography (Figure 34).

Lamp: (1) Intensity should be great enough to give a sufficiently bright image on the ground glass to permit accurate focusing. (1a) Since the source of light used for focusing will necessitate an extremely short exposure time when used with most negatives, a filter should be placed between the light source and the microscope. For example, if the correct exposure is 1 second it is more difficult to time it accurately than if it is 10 seconds. At 10 seconds the exposure time can be so varied that just the correct negative density can be obtained. It is possible, of course, to achieve this decrease in intensity by using resistances or neutral tint filters. However, by using a colored filter (usually green) an additional effect is obtained in that by using monochromatic light whatever chromatic aberrations are present in the optical system are eliminated and a sharper image will be obtained.

A shutter or other light-stopping arrangement (1b) should be placed between the lamp and microscope. The lamp switch may be used for timing the exposure if an incandescent filament bulb is being employed. (See "B 12′".) Where a roll-film camera is attached to the microscope, the camera shutter commonly is used, although this arrangement of the shutter being on the microscope or film-carrying mechanism introduces vibration.

The mirror (2) should be in alignment with the lamp and the rest of the optical system including condenser (3), objective (6), film holder (13). Microscope adjustments for condenser focusing, condenser diaphragm stop, and focusing of the objective is done according to the same theory as with visual microscope work.

Movements of the mechanical stage (4), the fine adjustment (7) and usually the coarse focusing adjustment (8) should be connected to extensions so that they can be manipulated while the operator is observing the ground glass (13).

The object must be mounted in a clear, stable medium (5), as mentioned earlier.

Choice of an objective (6) must be along very critical lines. While the equipment used is similar to that used for visual work, all effects are accentuated. Where apochromatic objectives and compensating oculars (9) give an improved visual picture, they are mandatory for photomicrography. Magnification can be obtained either by use of higher power objectives or oculars or by bellows extension. Oculars should be not over 10X. From 100–200X a 16-mm. objective should be used; from 200–300X an 8-mm. objective; at 400X a 4 mm. objective, etc. Some variation from these general figures will be encountered, depending upon the whole optical system.

It is necessary to provide some sort of a light trap (10 and 11) to connect the ocular to the bellows base plate and yet provide for free movement of either microscope or bellows each independently of the other. Attachments may be fastened directly to the microscope by means of the ocular (9′), which holds the attachment in place when it

fits into the body tube. When a camera is used, the bellows (12′) will have an approximate range of from 1 to 8 inches, but if a separate bellows unit is used (12) it should stretch from about 3 to 30 inches.

At the top of the bellows the image is projected on a ground glass (13) which is replaced by a plate holder or film holder (13′) when the exposure is made. Final focusing is best made while sliding the glass to eliminate the sandy appearance which is introduced by the glass. A camera attachment is focused according to the requirements of the particular camera involved.

On a permanent setup the bellows should be mounted so that its height and length are adjustable. The clamps (14) usually operate on a double or triangular track so that they cannot pivot out of alignment. The whole should be supported on a ridged column (15). Many types of films or plates are satisfactory but usually they should be relatively insensitive to red light so that their development can be observed in a photographic darkroom.

Up to this point we have assumed that a photograph is being taken to resemble the visual field as seen through a compound microscope and with a magnification somewhere between 50 and 1000X. This, of course, is unlike the appearance of objects under the Greenough binocular microscope and the field of view is relatively small. It is possible to take pictures through the Greenough type binocular but its use for this purpose is to be avoided. Resolution is poor. For low power photomicrography of opaque objects an objective similar to an ordinary camera objective should be used. In fact with the proper attachments a well-corrected camera objective is entirely satisfactory, although the optical companies put out especially constructed and mounted objectives for this purpose (e.g. Micro-Planars, Micro-Tessars, etc.). For a wide range of work, two or three objectives are ample. The 20 mm. objective can be obtained in a "society thread" mount and so used on a compound microscope. For lower power work a 32 and 50 mm. are more often used. The photography of opaque subjects closely resembles ordinary portrait work. The subject is so illuminated as to bring out whatever details are desired, the lens focused, and a picture taken. The camera also is similar to a portrait camera and consists of a lens, bellows, and film-carrying apparatus or plate holder, the only difference being that the bellows extension is much longer and should reach at least 36 inches. Usually illumination by diffused light from two sides will provide even illumination over such an object as a fish, insect, or leaf. A shutter is of little importance, for the exposure can be timed by uncovering the objective or switching the light on and off.

This type of photography has one pronounced advantage over that utilizing microscope objectives and eyepieces—the objectives contain iris diaphragms that may be stopped down to increase the depth of focus so that the whole subject is in focus. Many photographers who make use of this manipulation do not realize that when a lens is stopped down and when length of exposure time is no factor, the objective lens should be closed as far as it will go. This is because in general the aberrations inherent in lens design are best corrected for light passing through the center of the lens and when stopping down the lens, full use is made of this factor.

IX. FILTH RECOVERY METHODS

The types of filth and decomposition most commonly encountered in food and drug products examined by microanalytical methods are usually insoluble pieces of insects, rodent filth, or molds. Certain soluble constituents of urine may be detected by microchemical methods and a relatively insoluble substance such as uric acid from bird or insect excreta may be precipitated out and its identity confirmed, but the majority of substances which concern us here are pieces of plant or animal material. Occasionally glass and sand or quartz are encountered and these usually require specialized examination. (See section on Crystallography.)

Fortunately insect fragments, rodent hairs, and molds will stand rather vigorous treatment and still retain their microscopic characteristics although in strong alkali the hairs will dissolve and be lost. Where possible, then, the most satisfactory way to recover filth from food is to retain it on a filter paper after dissolving the food and filtering it. Such a process is possible in only a very limited number of cases. Sugars, some hard candies, and a few other preparations such as dextrose-malt preparations fall into this category.

By far the great majority of separations are made by some procedure involving a differential of wetting, specific gravity, size, solubility, and/or appearance of the filth and the food involved. Quite often a combination of steps is necessary before a satisfactory result is obtained. As is the case with chemical analyses, each precipitation or extraction is incomplete to some small extent and the fewer the manipulations involved, other factors being equal, the better the recovery.

Heavy material such as sand can be retained as a sediment by using heavier-than-water organic solvents with such material as peanut butter and ground spices. Canned leafy vegetables can be floated in strong salt solutions while adhering soil settles out. In either case it is necessary to work the soil free from the plant material, for when the bulk of the separation rises to the top there is a tendency for small particles to become entrapped and consequently not settle out.

A. Sedimentation in Heavier-than-Water Liquids

This procedure can be used to separate rodent excreta pellet fragments from cereals. In a liquid with a specific gravity near 1.49 the pellet fragments tend to settle out while much of the cereal floats. As the specific gravity is raised, more cereal is floated but some pellet fragments also will rise and be lost. It is necessary to strike a practical balance between the need for floating the plant tissue with the possible loss of excreta. Factors other than density play a part in this separation. The particles must be soaked in the liquid long enough to become fully permeated. The density balance is quite delicate and the containers should be covered and otherwise handled to avoid strong convection currents. The separations cannot always be completed in one operation and rather than repeatedly transferring from a separatory funnel to a beaker and back to a funnel it is easier with the cereals simply to carry out the separation in a beaker and gradually remove the plant tissue with successive decantations.

B. Sieving

Where practical, a size separation offers a quick means of separating filth from food. Adult insects, larvae, insect eggs, and excreta, as well as rodent excreta, bits of metal, etc., may be sifted from foods or a food-filth separation may be accomplished by washing the food (or filth as the case may be) through a screen leaving the filth (or food) retained on the screen. A screening operation can be used for a preliminary separation before other operations are carried out. For example, in a candy mixture containing chocolate and nuts, the nuts can be removed immediately and once they are out of the way, further operations are simplified. Sifting often is useful for a rough qualitative separation to give some indication of the advisability of performing a more time-consuming quantitative segregation.

C. Use of the Wildman Trap Flask

For most foods neither a plain water solution nor sifting will give the necessary separation and other procedures are used. Some time ago it was found that when gasoline is mixed with a watery mixture containing insects or insect fragments the insects float up with the gasoline layer. This principle has been utilized repeatedly for filth extractions.

1. Specific Gravity; 2. Oil Wetting.

Apparently part of this effect is brought about when the hydrophobic and oleophilic insect cuticle is wet by oil, which is lighter than the liquid in which the food is soaking, and, as the oil droplets rise, the insects are carried up. Some of the oil wets the smooth insect cuticle and some clings to external processes and, as shown in Figure 35, water is repelled. The extraction effect is similar to the extraction by ether of a substance suspended in water. Most plant tissues will settle out. This simple explanation is only part of the picture, for in some instances the separation appears to be due mainly to the natural lightness of the insects. This is particularly noticeable when the insects or fragments are dried out and contain air. Both the relative densities of the liquid, plant material, and insects and the increased differential caused by the oil increment are utilized in the flour method where the insects are floated from white wheat flour in a saturated-salt medium aided by gasoline.

3. Alcohol Penetration of Bran.

Bran reacts somewhat similarly to the insect tissues and floats into the upper oil layer. This difficulty can be overcome, in part, by substituting a water-alcohol solution for the plain water. The alcohol reduces the specific gravity of the solution and it also wets the bran better than does plain water so that it soaks into the bran, driving out the air. There is some indication that oils in alcohol-water solutions wet the insect fragments better than oil in water, although any increase in the wetting of the plant material by the oil is offset by the other factors.

4. Detergents.

Better wetting might be obtained through the use of detergents, although so far in practice it has not been found that they can be used to obtain a sharper plant separation or a more complete recovery.

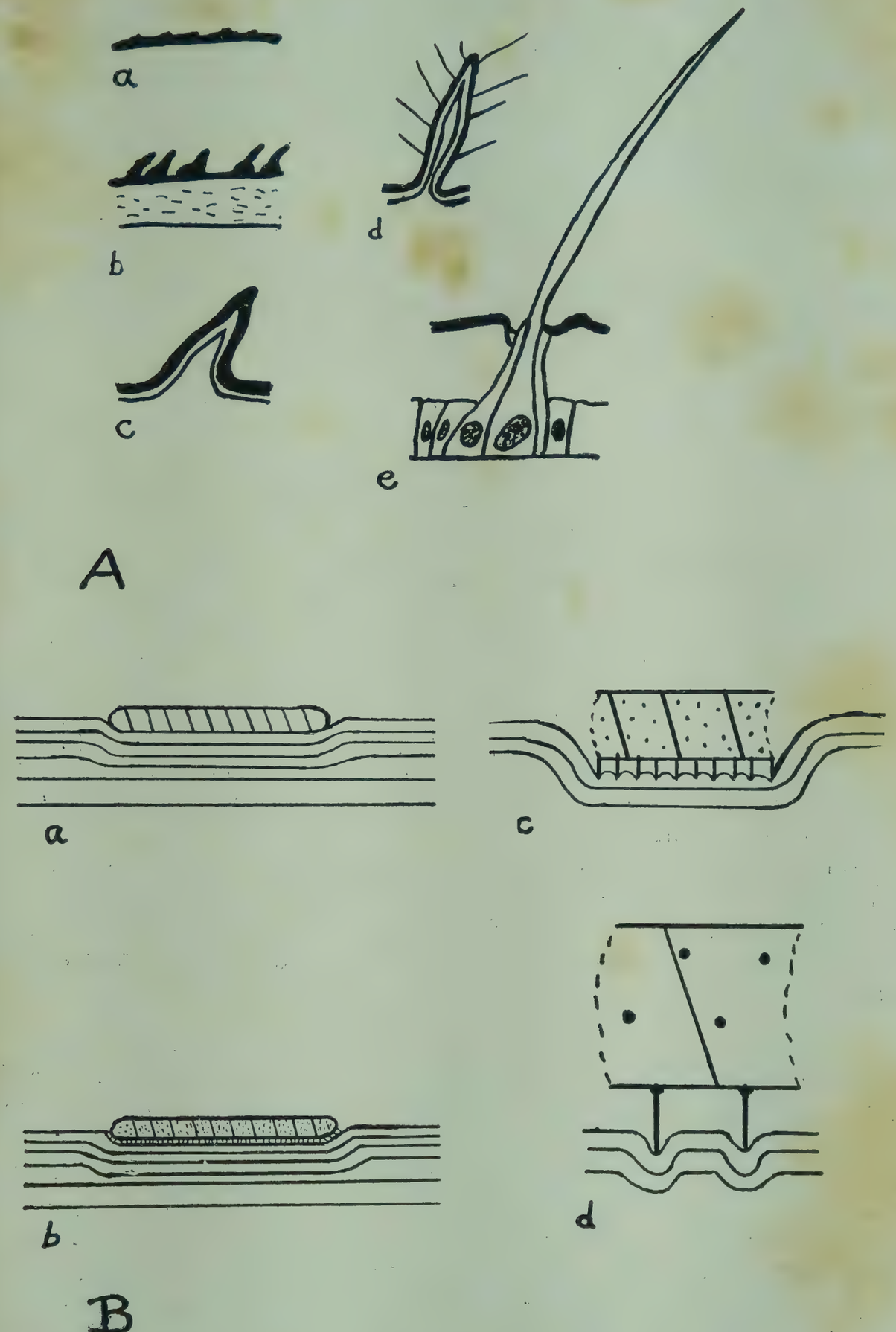


FIGURE 35.—The integument. *A*, Types of external processes: *a*, Non-cellular cuticular pattern; *b*, non-cellular cuticular spines; *c*, Spine; *d*, Seta; *e*, Elongate seta or hairs. *B*, Insect cuticle in contact with water: *a*, Smooth insect larva supported by the surface tension of water; *b*, Spiny larva as in *a*; *c*, Enlarged section of *b*; *d*, Section of *b* further enlarged. (*A*. From Snodgrass, Principles of Insect Morphology. By permission of McGraw-Hill Book Co.)

5. Boiling.

Troublesome deposits of starch or bran in the oily layer can be prevented by boiling before the oil is added, unless there is so much starch that boiling forms a considerable gel. At any rate, boiling drives out air and saturates the bran or chaff tissues with water so that there is less tendency for them to float in the oil layer. When an abundance of starch is giving trouble, boiling usually is to be avoided since it gelatinizes the starch. This gel then forms an emulsion when the oil or gasoline is stirred into the mixture and it is impossible to obtain a clean separation unless, as will be discussed later, additional digestion processes are used. Starch in a mixture which is being extracted in a Wildman trap flask is best handled in cold water, although usually when starch is present, bran is also, and alcohol solutions are used.

6. Emulsions.

We are just beginning to appreciate all of the factors concerned in the oil flotations of filth. The ultimate aim, of course, is to float the light filth fragments and still allow the plant material to settle out. In practice this separation is incomplete because of the conditions noted above and several additional factors. It is difficult to wet all of the insect material with oil without creating a frothy emulsion. The emulsions formerly were thought to be troublesome only because they included plant material which obscured the filth in subsequent microscopic examinations. Further experience has shown that, in addition to this, the fairly stable emulsions can hold filth down in the trap flask and out of the neck of the flask and once the emulsions form it is difficult to break them and set the insect fragments free. At times emulsions may be broken with a few drops of ethyl or caprylic alcohol. The oil or gasoline should be worked into the food mixture with as little inclusion of air as possible and intermittent agitation should be provided while the separation is taking place. Persistent emulsions sometimes are caused by the release of dissolved air from the water, and, because of this, de-aerated water is recommended for extractions in a Wildman trap flask.

7. Oil Types.

Both the formation of an emulsion and the wetting of the filth and/or plant material by the oil are dependent upon surface phenomena which will vary with any of the components of the mixtures or solutions. Light oils such as gasoline form more troublesome emulsions as a rule than do light and medium mineral oils, although if an emulsion once forms with the heavier oils it is very difficult to break. The oils most commonly used are gasoline, kerosene, mineral oil, and castor oil. Castor oil is heavier than the other oils yet it will carry insect fragments up with it as it floats, but because of its density it must be used in water. Also, it is very viscous and its use so far has been confined to hot mixtures. For some reason, rodent hair recoveries are poor when castor oil is used.

8. pH.

Some recent work has confirmed the surmise that the acidity of the solution in which a gasoline flotation is being carried out has a significant

effect on the insect fragment recovery. At pH of 7 and above the recovery of insect fragments appears to be lower than from an acid medium.

9. Cleaning the Flask Sides and Dilution.

In addition to the oil-water interface, the side of the flask presents a very troublesome surface. Oil droplets, some of them holding filth, cling to the sides and they must be cleaned off repeatedly with the rubber stopper plunger. When oily liquids are used in trapping off filth they should be added only to a wet flask since, if added to a dry flask, they will wet the sides and filth may be held on the sides and not float into the upper oil layer. Since the filth must rise up through a mass of descending material the descending material must be dilute. In other words, both in Wildman trap extraction and in heavy filth sedimentations, crowding too large a sample into a small container will cut down on the filth recovery. It is necessary to stir the bottom (or top) layer to release entrapped filth fragments during extractions.

D. Maggot Recovery

Fly eggs and maggots (and at least some nematodes) respond differently to oil-water flotations than do other insects. They settle out while other insects float. Two theories have been advanced to explain this; both may play a part. While it is known that certain maggot parts (e.g. the spiracles) are hydrophobic and oleophilic it may be that the remainder of the maggot body surface does not attract oils. Then again the maggots encountered in foods live in a watery medium and perhaps they are simply too full of water and too heavy to be floated. Because of their peculiarities they are separated by a process opposite to that used for other insects and insect fragments. They are permitted to settle out while the plant material is floated. Consequently, instead of gently stirring gasoline into the mixture, as is carried out in a Wildman trap, the mixing is carried out vigorously in a separatory funnel so that the plant tissue is caught in the rising gasoline and air and carried up and away from the maggots and eggs which settle. Since even small fly eggs and maggots can be retained on a 10XX bolting cloth, a mixture containing such filth can be filtered on the cloth rather than on a filter paper. By its use much of the extraneous matter is lost through the cloth and the microscopic examination is simplified.

Maggots may be recovered by other sedimentation procedures which are somewhat similar to the "panning" or washing for gold. Pulped fruits, jams, etc. sometimes are handled in this way, one method being to dilute the food with water and stir it into a wide pan. Then after the maggots have settled out, the top water mixture is poured off so that the maggots are retained in the lower corner of the pan.

E. Removal of Undesirable Components Before Extraction of Filth

The above-discussed sedimentation, sifting, and flotation of filth constitute virtually ideal conditions from the analyst's point of view. In practice many foods have idiosyncrasies which make it necessary to

examine each by an individual procedure. Some mention has been made of the trouble bran and emulsions cause. At times the troublesome elements become so acute that they must be removed before a filth extraction can be attempted.

1. Starch and Protein Digestions.

Starch can be hydrolyzed by different methods. Boiling, boiling in dilute acid, or enzymatic digestion all have been used. Proteins can be broken down by comparable methods and they along with starch are handled with pancreatin. Enzymatic digestions have been most helpful with bakery products where the enzymes are used to release filth from the food and to produce a liquid mixture which can be extracted or filtered. The digestions are accomplished readily if the right conditions are maintained. Pancreatin has been the most useful enzyme and its pH and temperature requirements are rather easily fulfilled while it acts on carbohydrates, fats, and proteins. It sometimes is necessary to boil or otherwise soften the food to prepare it for digestion but at other times a dilute pancreatin solution can simply be soaked into the food and digestion will start immediately.

2. Fat and Oil Removal.

Fats and oils are troublesome under some conditions and of minor importance in others. Chloroform or carbon tetrachloride used for the sedimentation of heavy filth will remove enough of the oil from corn meal, and some of the spices, so that any subsequent flotation for light filth can be carried out without further attention to the oils. In other instances petroleum ether should be used to remove fats and oils. Bakery products with large amounts of shortening can be digested with pancreatin more readily if most of the shortening is first removed, by soaking in an organic solvent and decanting off the oil-bearing solvent, to permit more complete penetration by water and the enzymes. This principle of oil removal is one of the simplest in the filth methods. At times, NaOH can be added to emulsify the oils and while they are not removed the soapy emulsion formed can be handled more readily than could the original droplets of fat.

3. Treatments to Avoid With Hairs and Insect Fragments.

Fortunately insect fragments, hairs, and mold will withstand rather vigorous treatment and yet retain their identity. However, hairs are susceptible to the action of alkaline solutions and strong alkalies such as NaOH or KOH when used hot even in 1-percent solutions will dissolve rodent hairs. Hence when rodent hairs are to be recovered the use of these and other relatively strong alkaline reacting substances such as Na_3PO_4, Na_2CO_3, and NH_4OH should be avoided.

Hairs are much more resistant to the action of acids, although the use of hot H_2SO_4 and HNO_3 at even 5 percent should be avoided. Rodent hairs will hold up for 15–40 minutes in boiling 5 percent HCl, the degree of attack on the hairs depending upon the protection given them by the particular food. H_3PO_4 and the other relatively weak acids are much safer. After treatment with acids the hairs may be soft or brittle and should not be subjected to vigorous mixing action. On a

filter paper they cannot be teased or turned readily without breaking. A further difficulty introduced by severe acid treatment is that the characteristic morphological pattern may be altered somewhat and in identifying hairs by the NaOH swelling technique the treatment they have been subjected to must be taken into account since a characteristic pattern may not be obtained. It is believed that rodent hairs processed in acid foods, such as tomatoes, apples, and grapefruit, give a slightly modified pattern when swollen in 10 percent NaOH as compared with the unprocessed hairs.

Insect fragments will withstand any chemical action to which it has been found necessary to subject the food. Alkali will clear insect fragments and remove much of the pigment, especially when used in strong concentrations but if the treatment is taken into account even fragments boiled in saturated aqueous KOH can be identified as insect parts almost as readily as could the original untreated pieces. However, it must be remembered that strong NaOH or KOH softens insects and after boiling in aqueous 10 percent NaOH for 5–10 minutes they are more subject to mechanical breakage. For all practical purposes in filth work, insect fragments and insects may be considered to be unaffected by acids, even though they can be carbonized by extended boiling in strong H_2SO_4.

Violent mechanical stirring or grinding of foods should not be used for filth extractions. Tests have demonstrated that rodent hairs agitated in a liquid by a malted milk stirrer sometimes survive unbroken and sometimes are broken. Inasmuch as there is no way of determining what the effect will be in the particular sample being examined, the stirrers should not be used for any quantitative analysis. Insects are broken by such stirring. When the insects are dry and large the damage is greater than when they are pliable and small. Dry grinding comminutes the filth even more than does wet agitation.

F. Solution and Filtration

Sometimes it is possible to complete a separation by simply dissolving off the food and leaving the filth in a beaker or on a filter paper without resorting to any further treatment. Chewing gum can be handled in this manner. Boiling in dilute acid solution will dissolve the water-soluble portions and also hydrolize a starchy coating or dissolve any carbonates that may be present in the coating. The acid boil also serves to soften and disperse the gum so that it is accessible to other reagents. However, the boiling must be stopped before carmelization of the carbohydrates has gone too far. Acetone can be added to the water to facilitate the solution of the chicle component by an organic solvent such as turpentine or chloroform. By attacking each component separately it is possible to filter the gum through a No. 100 or 150 sieve or bolting cloth.

G. Washing the Filth from the Food

Washing is obviously advantageous with such products as nut meats and dried fruits where either plain water or water and detergents can be used to remove and concentrate a surface contamination for microscopic observation.

H. Centrifuging, Etc.

On occasion centrifuging may solve an otherwise difficult separation problem. In general, procedures involving few transfers of material and few manipulations will give better recoveries than more complicated methods. Each manipulation introduces a point at which material may be lost. This does not mean that a separation in a 100–ml beaker can be more efficiently carried out than in a 400–ml beaker, for at times it is necessary to dilute the menstruum in order to loosen adhering filth. Whenever a method is under question it should be checked by repeated runs with known amounts of material added.

I. Oil Clearing and Sedimentation for Excreta[2]

There are two general methods by which insect excreta in flour may be counted. One utilizes sedimentation of the excreta in a heavier-than-water liquid, which floats the flour, and the other consists in so treating the flour that the excreta are made visible in the matrix.

Several difficulties may be encountered in flotation procedures because different particles of flour vary in their specific gravity, depending upon the part of the grain from which they were taken. For example, the bran is heavier than the starch, and even similar parts vary in weight according to the presence or absence of entrapped air or other factors. Insect excreta pellets similarly show a wide density range depending upon the type of food eaten, physiological condition of the insect, and the species or stage of the insect. A liquid with specific gravity 1.52 will support most flour particles, but in it many insect excreta pellets may rise into the flour layer. At a specific gravity of 1.40, the insect pellets settle out quite well, but much floury material settles with the excreta. Because of these difficulties it is impractical to depend solely on specific gravity for separation.

Insect excreta pellets behave differently when moistened with an oil than do wheat flour granules. The latter tend to become clear and transparent when immersed in an oil medium, while the excreta pellets tend to retain their white, opaque appearance. The refractive index is important in rendering flour particles transparent; with higher refractive indices the flour is less apparent that it is with lower indices.

Factors other than refractive index must be considered in making the flour transparent and the pellets visible by contrast. The oil must readily penetrate the flour and drive out most of the air, and it must not be too volatile or the prepared mounts will be unstable while an examination is being made. In actual practice clove oil has been found to satisfy the requirements of such an examination although with any of the oils certain phosphates and salts found in self-rising flour remain opaque in oil and may bear a superficial resemblance to fragments of excreta pellets while some fragments of cereal fail to clear completely and so resemble excreta. Mineral material will appear crystalline unless its crystals have been destroyed by grinding, but in this case the appearance will be dissimilar to insect excreta in that the excreta will be smooth and rounded while the minerals will be angular and have flat or irregular cleavage sides. Internally the excreta show a heterogeneous laminated appearance while the salts are homogeneous.

[2] From Jour. AOAC **26:** 257 (May 1943).

In examining a product for whole rat or mouse excreta a simple visual examination may be used, or sifting employed where necessary. Where an oil flotation of a product is carried out in a Wildman trap flask the rodent excreta will be found settled to the bottom with the other heavy matter.

With comminuted products, for example flour, meal, spices, and crude drugs, it is advisable to float off most of the food or crude drug material so that the rodent excreta is concentrated. Such a separation may be obtained by using an organic heavier-than-water solvent such as choloroform (specific gravity 1.498). In such a solvent most of the plant material will float and the rodent excreta pellet fragments will settle out. Where trouble is encountered because too much of the plant material settles out with the rodent excreta, the specific gravity may be raised by the addition of carbon tetrachloride (specific gravity 1.595) to the chloroform but the specific gravity should at no time go above 1.546 (chloroform to carbon tetrachloride 1:1) because at this point the rodent excreta fragments may rise and be lost in the floating layer. Such separations may be carried out in a separatory funnel, crude drug percolator, or simply in a beaker, depending upon the nature of the particular substance involved. In tea, for example, where all of the leafy material readily floats and the particles are not finely ground, the separation can be carried out in a crude drug percolator, but when repeated decantations are necessary and the product is not so finely ground that a separatory funnel can be used, sedimentation can be carried out in a beaker and the heavy filth gradually concentrated at the bottom of the beaker.

X. EXAMINATION OF WHOLE FOODS FOR GROSS INSECT AND RODENT CONTAMINATION

A. Insect Chewing, Tunneling, Webbing

The filth extraction procedures represent the best available means for separating the tangible evidence of filth from a food so that it may be examined and counted. If insect, rodent, or mold contamination can be judged adequately by a visual examination of the untreated product such as a consumer would use, this means should be employed. Whole or dried fruits, nuts, alimentary pastes, candy, etc., are the type of foods that can be examined in this manner.

At times insects may be swarming over the product and a cursory examination will suffice, but usually the damage will be less obvious and it is necessary to examine each piece with considerable care, although still on a semi-macro basis, to determine the presence or absence of insects or insect-damaged pieces.

Insect tunneling and chewing is quite typical; it is often accompanied by an accumulation of chewed bits of the food, insect fragments, and insect excreta commonly called "frass." The presence of frass is one character that may be used to distinguish cracks and blemishes from infested areas. In Figure 36 there is no eaten area apparent in the interior surface of the date but the insect excreta is visible. Walnuts may be infested while the nut is immature and still growing; consequently the worm-cut areas turn dark and show a pronounced discoloration. (Figure 37.) However when the insect-eaten areas are open, accessible pits, the frass usually is removed in cleaning. This is particularly true in such cases as cut green beans (Figure 38) where the

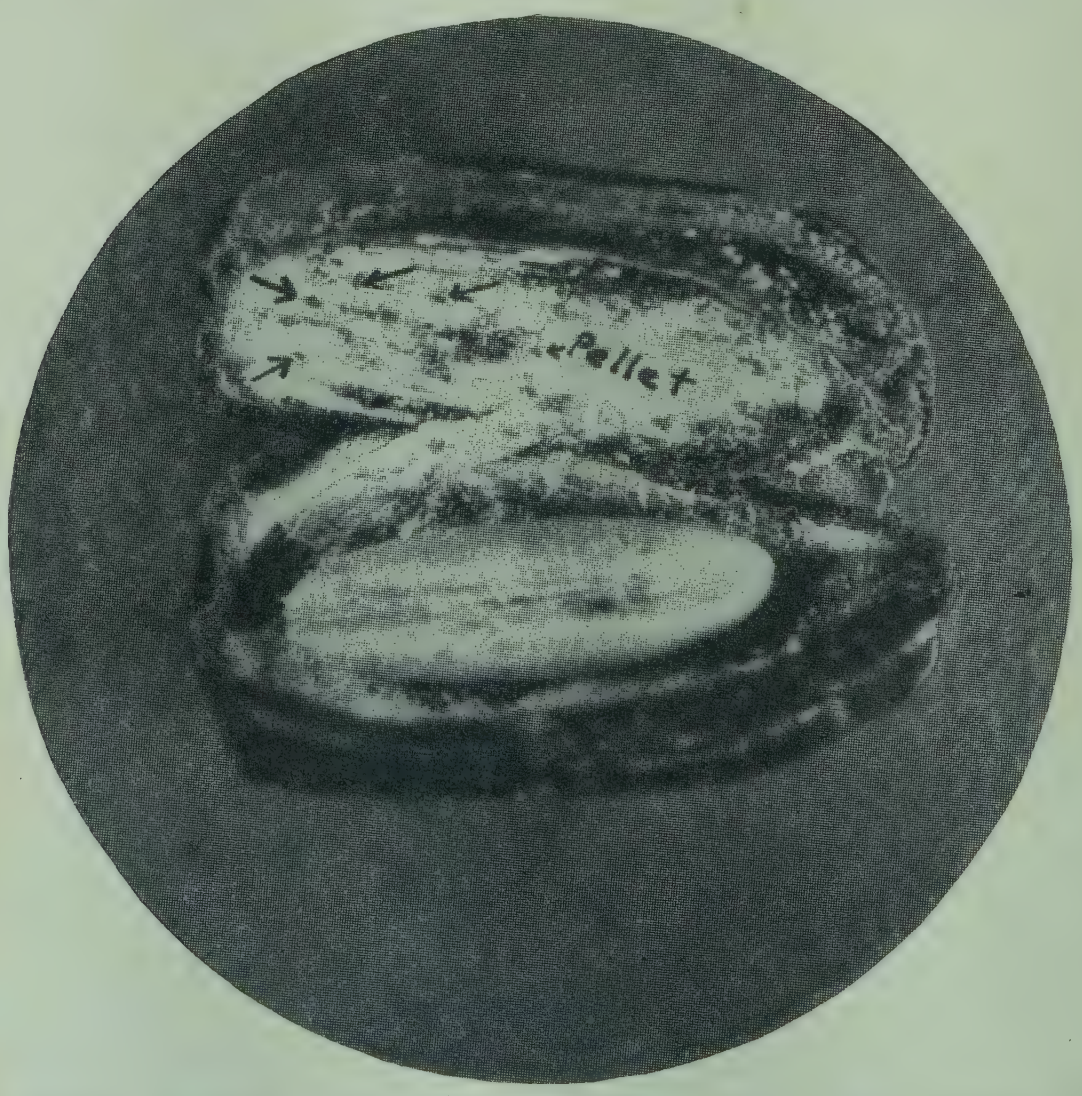


Figure 36.—Date showing excreta from Indian meal moth larva. The excreta appear as dark specks when the fruit is cut open.

somewhat rounded edge-darkened "craters" remain, but usually the frass has been washed out. Note also that Riker mount I–1 of orchard-damaged walnut meats and I–2 of storage insect infested almonds show the darkening characteristic of damage to the growing plant and the undercut and rounded edges characteristic of all insect feeding. This feeding is in marked contrast to irregular chipped or mechanically damaged areas. Tunnels through the material may follow either an irregular or straight course. The tunnels themselves are approximately circular and may have enlargements and dead-end branches where the insect paused to feed. Some insects literally honeycomb the food with their galleries. Others eat into an area and stay in one place eating out one large chamber. Where the feeding is along tunnels in the food, the diagnosis based upon frass, or the presence of the adult or larva insect itself, is rather simple, but surface feeding, in which the frass is less firmly retained, may be less readily separated from mechanical chipping or cutting. Insect-eaten areas usually are rounded crater-like cuts where the insect stood in approximately one place and fanned out in its eating in various directions. At times insect feeding is selective, so that the germ of seeds may be eaten before other areas are attacked. On green beans the insects will cut deep pits but the asparagus beetle eats in a shallow expansive pattern, not going much below the epidermal layers.

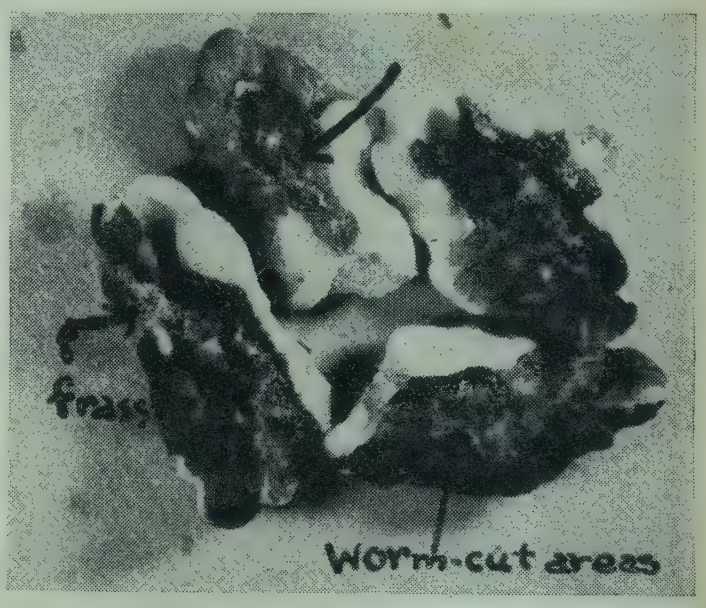


FIGURE 37.—Walnut meats infested in the orchard with coddling moth larvae.

The pattern of webbing will depend upon the insect and the food. It may trail endlessly all through the food, matting it together in a loose conglomerate; or it may appear, for instance, on individual nuts as a fine cottony covering; or it may be in the form of silken tubes (Figure 39). In any case, in granular or powdery material, such as flour or meal, there will be an accumulation of particles on the web so that in comminuted cereals it is not the web which is readily visible but the material held to it.

The exhibit material should be studied with considerable care so that the differences between mechanical injuries and insect-eaten areas are clearly understood. Indentations along the edges of nuts should be viewed with caution since insects are most likely to work down in protected areas and a nut, of course, could very readily be chipped along an edge as in the case of shelled peanuts and almonds. At times a rounded cut, in which dark particles of dirt have become lodged, will appear to be an insect cut filled with frass. A little more than casual scrutiny will resolve the difference between bits of dirt and excreta. A more common source of error is to mistake tannin cells for insect excreta. Tannin occurs as inclusions in the cells of many plants and in certain tissues it may fill entire cells so that it appears as small, brownish-red granules that somewhat resemble pellets. It is this tannin which imparts the bitter astringent flavor to the septa of pecan nuts. Tannin cells have been mounted in slide I–31. An examination

FIGURE 38.—Wax beans damaged by the Mexican bean beetle.

FIGURE 39.—Insect webbing from cocoa beans. The webbing consists of seemingly "endless" filaments of fine colorless silk, amorphous in that there are no granules, cell walls, etc. within the hyaline filaments.

of this slide will show that these cells are translucent, glistening, homogeneous bodies with smooth outline. Some are round and others more ovate but none are heterogeneous and laminated, nor do they have the irregular outlines of insect excreta. (Note slides I–7, I–3, and I–R–31, and Figures 40 and 41). In contrast to frass, tannin cells turn deep blue when treated with ferric chloride.

Certain plant diseases, for example, anthracnose on green beans, form deeply pitted areas which are similar to pits that have been eaten out by insects. These may be differentiated by the presence of mold in the depressions, the dark discoloration, and the general shape of the damage.

B. Whole Larvae and Adults

Obviously where the whole insects are present the examination should be carried out so that their presence is noted. A discussion of the commonly encountered forms will be found in section XI, which discusses the insects themselves.

C. Rodent Contamination

To avoid repetition, a discussion of the examination for various types of rodent contaminants will be included along with the identification of those contaminants, as given in section III.

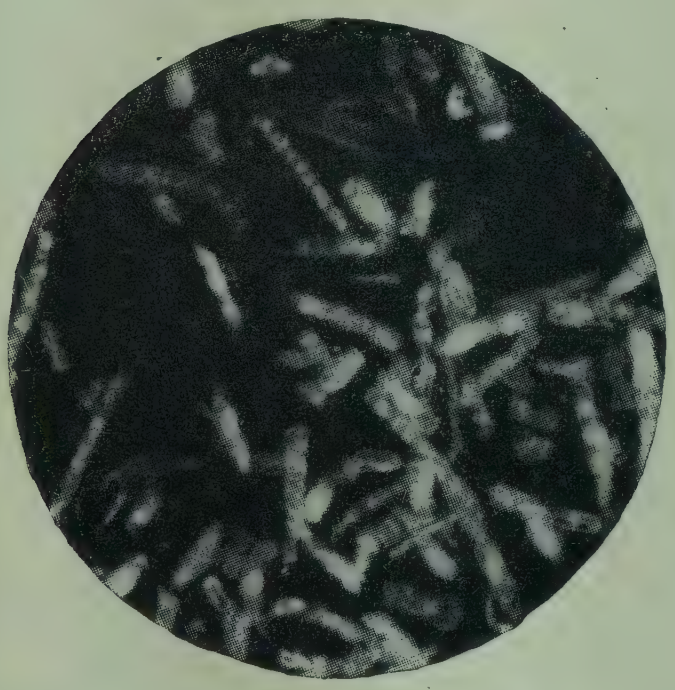


Figure 40.—Excreta from confused flour beetle in flour. Reflected light.

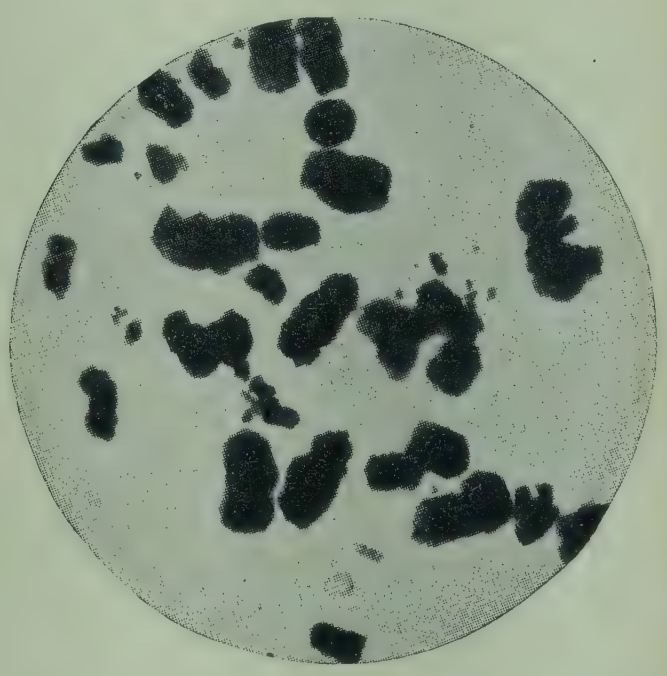


Figure 41.—Excreta from Indian meal moth larva in prunes. Transmitted light.

XI. INSECTS AS FILTH

A. Insect Morphology

Insects are divided into three body regions: Head, thorax, and abdomen, (Figure 42). At the anterior end is the head. The mouth is typically anterior and ventral but in some forms the mouth opening is at the end of a beak formed from the modified mouth-parts and is located near the neck or the thorax. The cockroaches (orthoptera) and buprested borers (coleoptera) have the head situated under the prothoracic shield so that from the dorsal surface the prothorax looks like the head, the head being visible only from the ventral aspect.

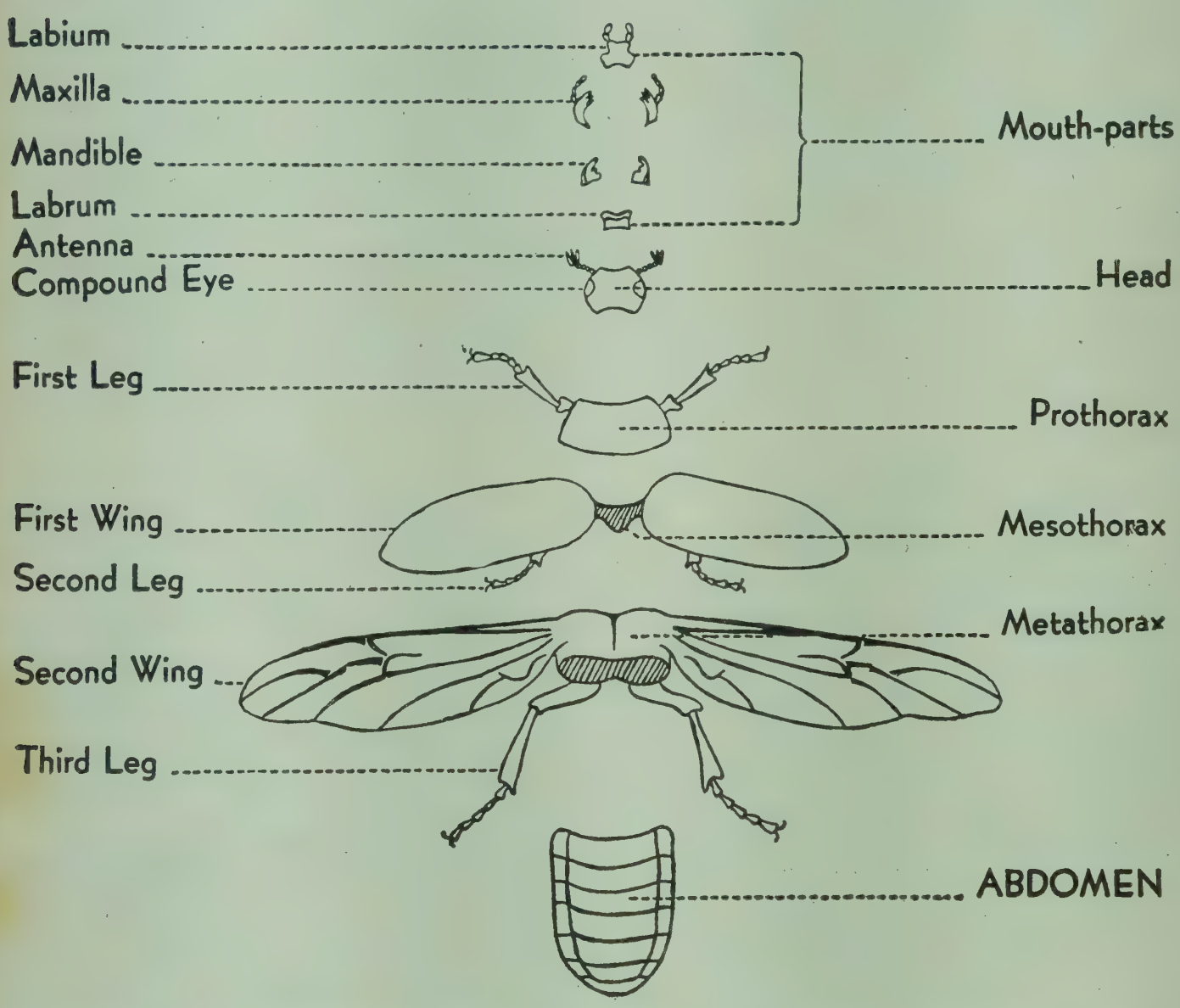


FIGURE 42.—Adult beetle separated to show parts. (From Minnesota Agric. Expt. Sta. Bull. 198.)

1. Head.

In larval insects (Figure 43) the mouth and its surrounding mouth parts are the most conspicuous head parts. The head itself is composed of a more or less rigid capsule which is roughly analagous to the vertebrate skull (Figure 44). Characteristically, there are two darkly pigmented mandibles and variously modified labrum, maxillae, and labium. Some of the parts are modified in some groups (lepidoptera) into feelerlike spinnerets.

Peculiarly the prominent adult compound eye does not occur in the larva although there may be several simple eyes or ocelli which usually are grouped in two dorso-lateral areas on each side of the head. The antenna is more or less prominent, sometimes appearing almost as a seta and at times being stoutly formed and several-jointed. The capsule sutures commonly are visible, one pronounced thickening originating mid-dorsally as the cranial suture and branching as an inverted "Y" into two frontal sutures which reach laterally and ventrally to the clypeus, resembling an upper-lip. Between the two frontal sutures is the frons. All of these structures are conspicuously present when insect larvae are comminuted in foods. The anterior articulations of the mandibles also occur near the lower end of the frontal sutures and figure prominently in insect fragment counts. Various setae and pigmented areas also occur. In addition to these external structures one rigid internal structure should be noted. Across the mid-posterior region of the cranium (in human anatomy it would be from ear to ear) is the so-called tentorial bridge which consists of a hollow tube with two barely noticeable external pits at each end and two branches anteriorly which terminate also in minute pits—these at approximately the same point as the ventral terminations of the two frontal sutures. This whole cross-strengthening construction has the appearance of an underlined "U" with the mouth situated between the open ends of the "U." Of the various soft parts, none are important in insect-fragment work.

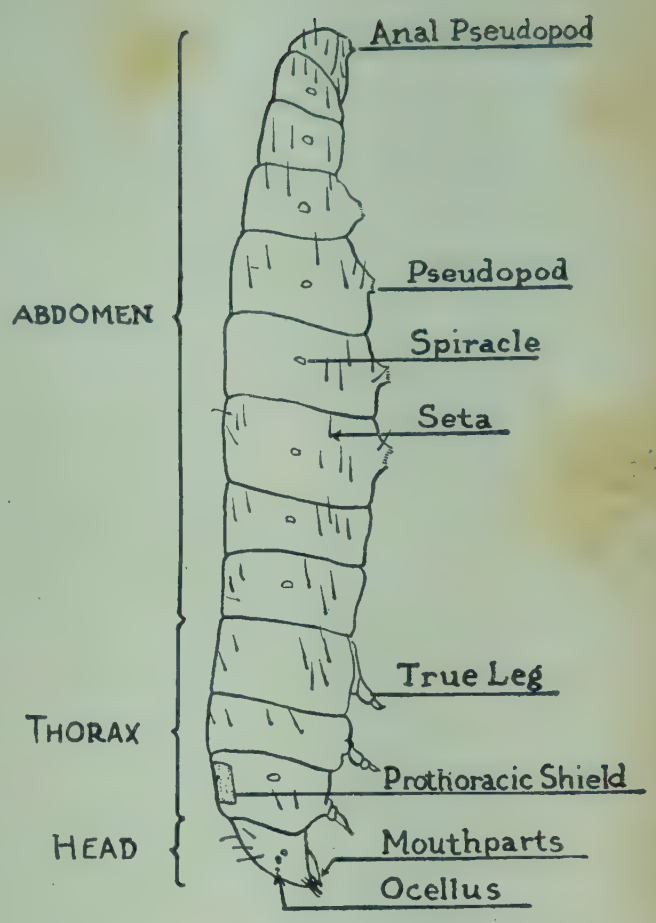


FIGURE 43.—Caterpillar.

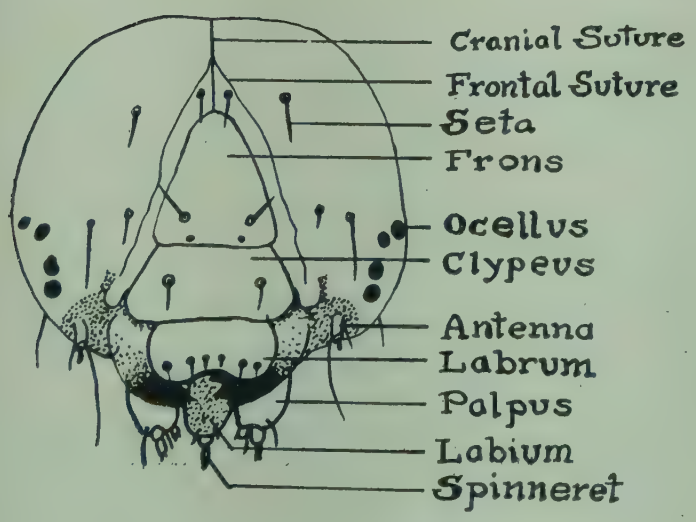


FIGURE 44.—Anterior view corn earworm larva (caterpillar) head.

The structure of the anterior end of most maggots is quite unlike that described above, since there is no head capsule (exception is the mushroom miner) with its accompanying eyes and external appendages. Most maggots are rather pointed anteriorly and contain the paired pigmented mouthhooks. The hooks are shaped differently in different species but are characteristically curved and somewhat toothed. They may be protruded or retracted and are used in feeding. They often are articulated against, or their musculature fastened to, paired basal plates.

The adult insect head is built along the same general lines, the antennae and eyes usually becoming more prominent. Ocelli when present are arranged in some orderly geometrical pattern. When three are present they form a triangle near the junction of the cranial and frontal sutures. The compound eyes are made up of many facets and in food contaminated with insects the eyes reach their ultimate in size in the flies. (See Slide I–R–35.) Antennae again are variously modified, as in Figures 47

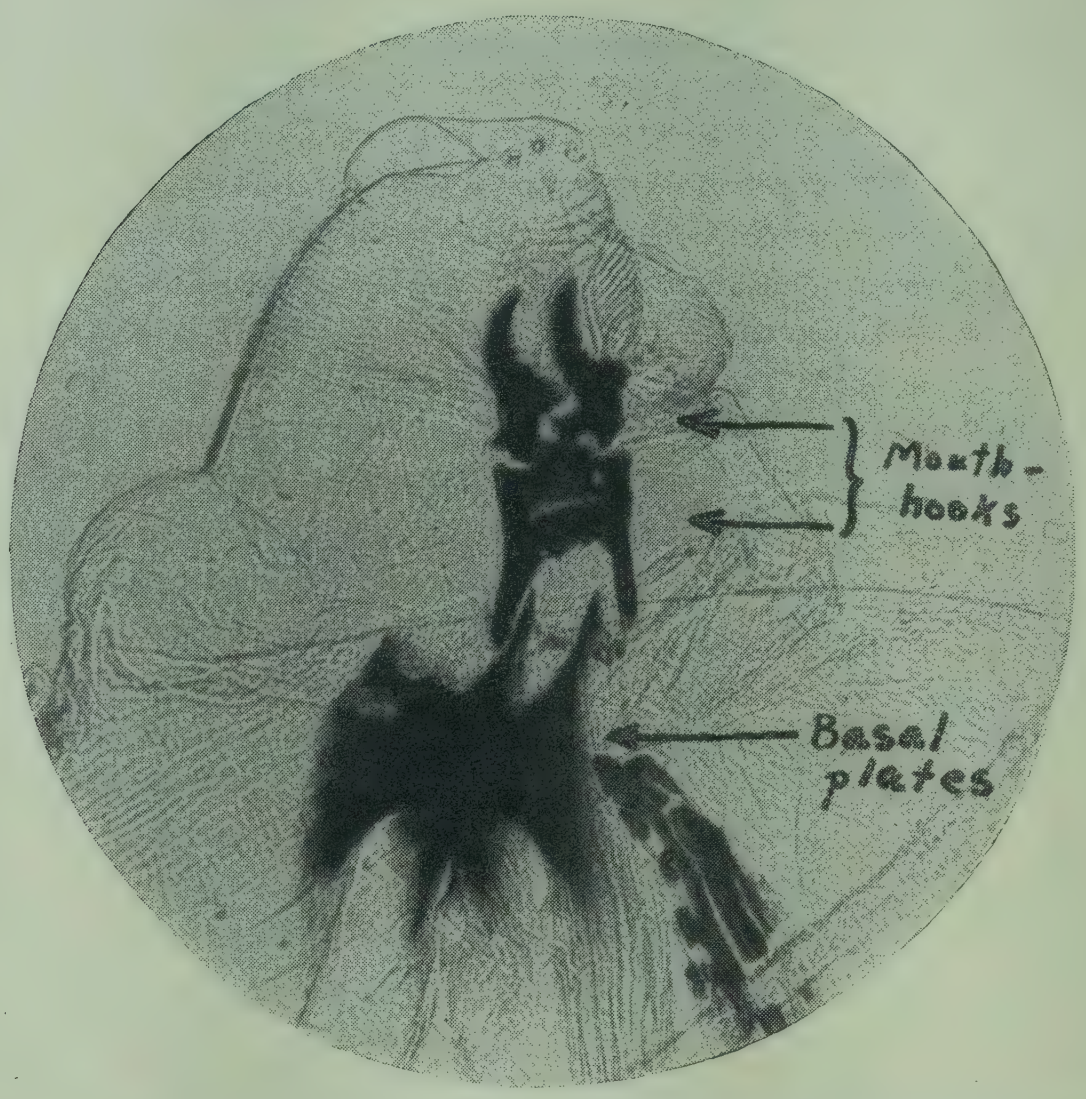


FIGURE 45.—Anterior end housefly maggot showing prominent dark mouthhooks.

and 48, and may be seta-like but more usually are conspicuous, elongate-slender, plumelike, club-shaped, laminated, toothed, etc., segmented protrusions sometimes naked and sometimes covered with setae. The antennae in certain groups are very characteristic. Mouth-parts too are variously modified and this will be discussed later in detail. It may be noted here that the adult male beetle usually has more ornate antennae than the female. The grasshoppers and cockroaches (orthoptera) have the most primitive type of insect mouth-parts, consisting of an upper and lower lip each formed from a flap-like extension of the head, a pair of mandibles, and a pair of maxillae, each with a finger-like palpus. Some mouth-parts are simply elongate-coiled tubes for sucking (lepidoptera), lapping and sucking (some diptera), piercing and sucking (homoptera, hemiptera, some diptera, etc.). In the curculios (coleoptera) the mouth-parts are situated at the end of a long, prominent beak or snout. When the basic chewing mouth-parts are modified into

a piercing and sucking type, the labrum and labium often form an enclosing sheath while the mandibles and maxillae form the piercing stylets.

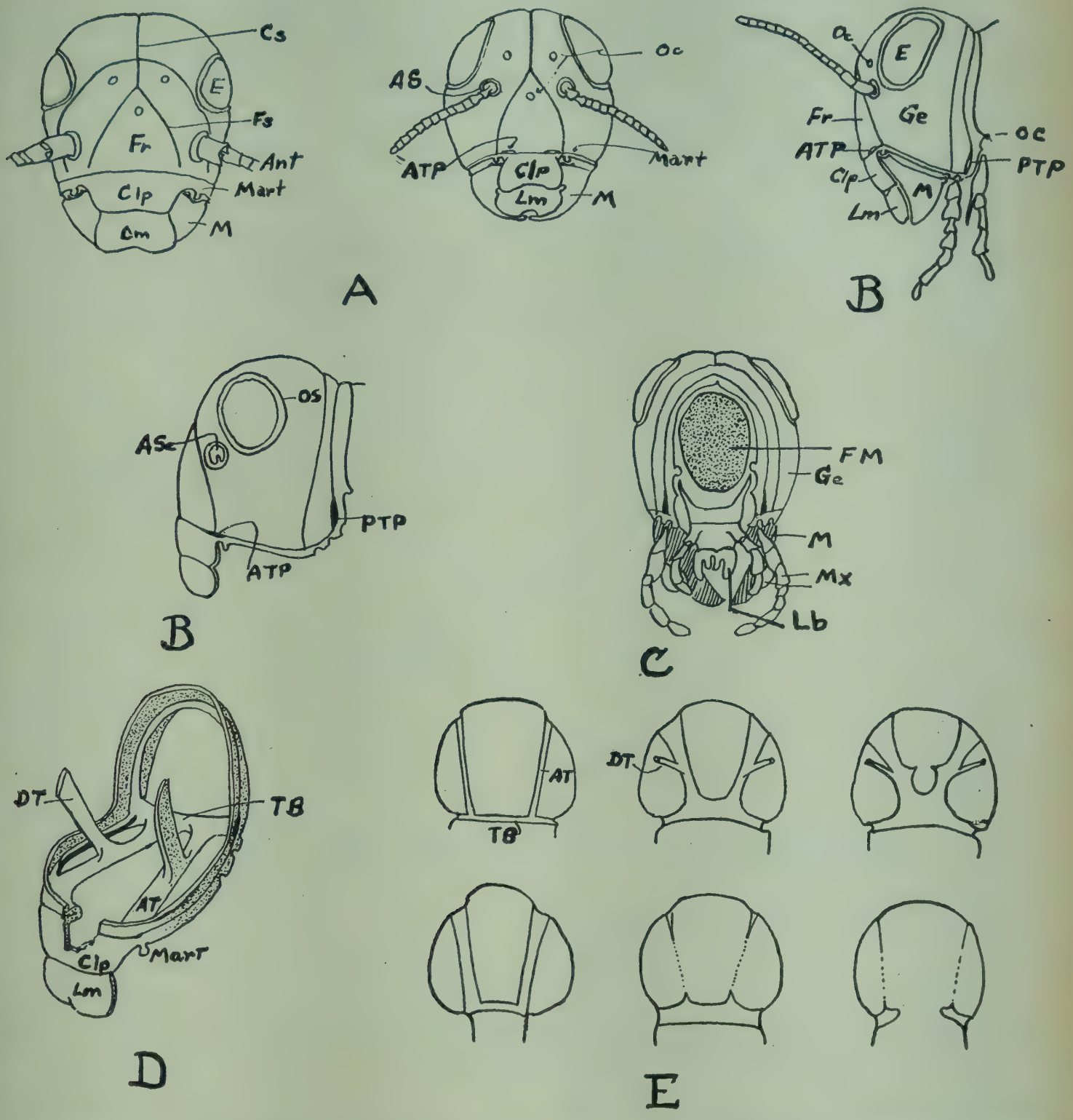


FIGURE 46.—The head. *A*, Anterior; *B*, Lateral; *C*, Posterior; *D*, Interior skeleton; *E*, Tentorium modifications. (From Snodgrass, Principles of Insect Morphology. By permission of McGraw-Hill Book Co.)

Key: Ant = antenna; AS = antennal suture; ASc = antennal socket; AT = anterior tentorial arm; ATP = anterior tentorial pit; Clp = clypeus; Cs = cranial suture; DT = dorsal tentorial arm; E = eye; Fr = frons; FM = foramen magnum; Fs = frontal suture; Ge = gena (or cheek); Lb = labium; Lm = labrum; M = mandible; Mart = mandible articulation; Mx = maxilla; Oc = ocelli; OC = occipital condyle; OS = occular suture; PTP = posterior tentorial pit; TB = tentorial bridge.

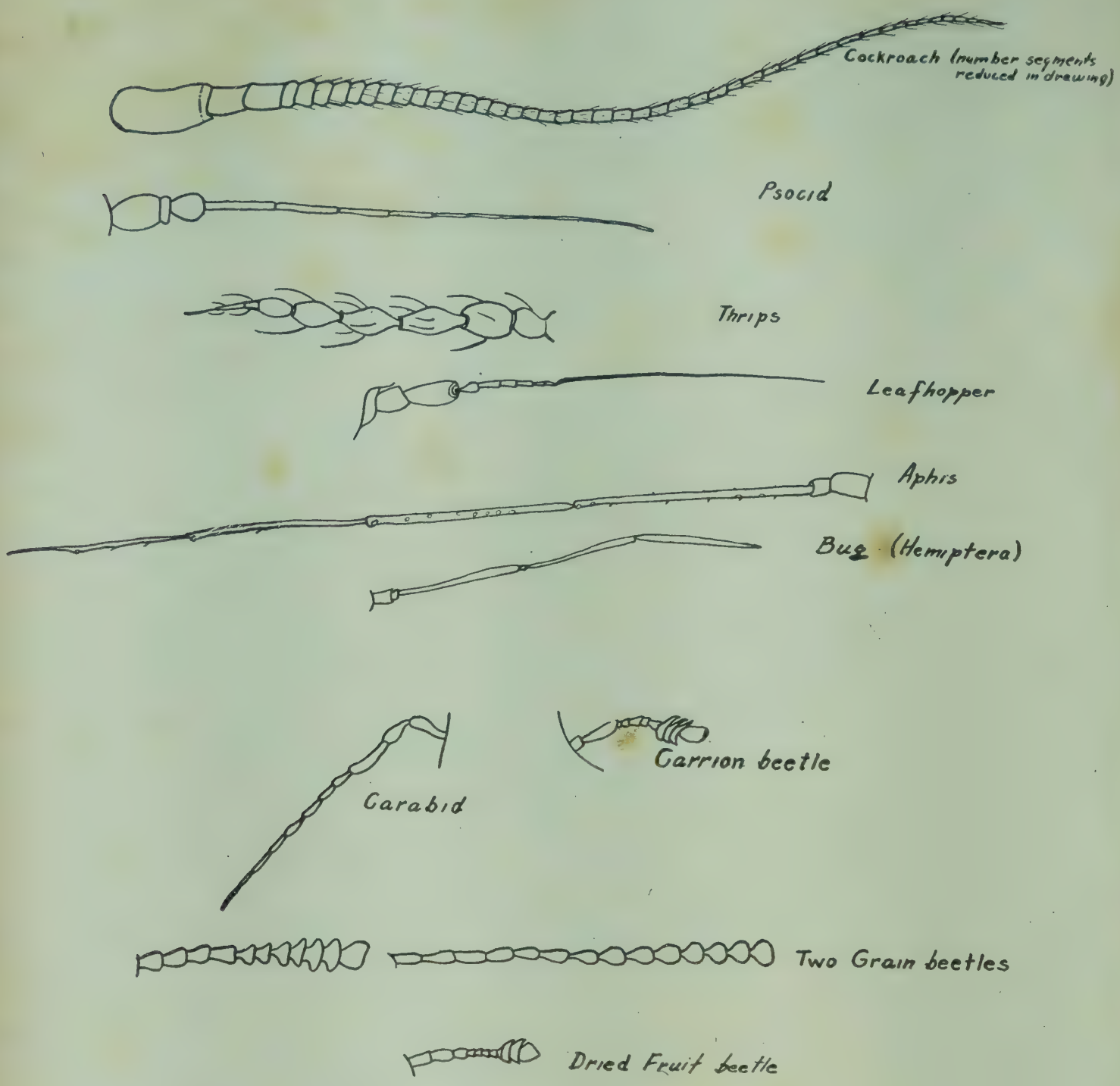


FIGURE 47.—Types of antennae.

2. Thorax.

The adult insect neck is sometimes a slender stalk joining the head and thorax. In some groups it is short and the head and thorax appear to be joined along a broad suture or the pronotum may extend dorsally over both the neck and head. In the larva, there is no neck. The head is joined to the thorax in the same manner as one body segment is joined to the next.

The thorax proper consists of three segments; typically, each adult segment bears a pair of legs (in larvae a true foot) and the second and third segments a pair of wings each. The thoracic segments, proceeding posteriorly, are termed the pro-, meso-, and metathorax. Each segment is formed from a complex group of interrelated, fused, overlapping, and anastomosing plates, of varying rigidity. The dorsal sclerite, or plate, of the most anterior thoracic segment is the large pronotum. It is this sclerite which forms the broad conspicuous shield

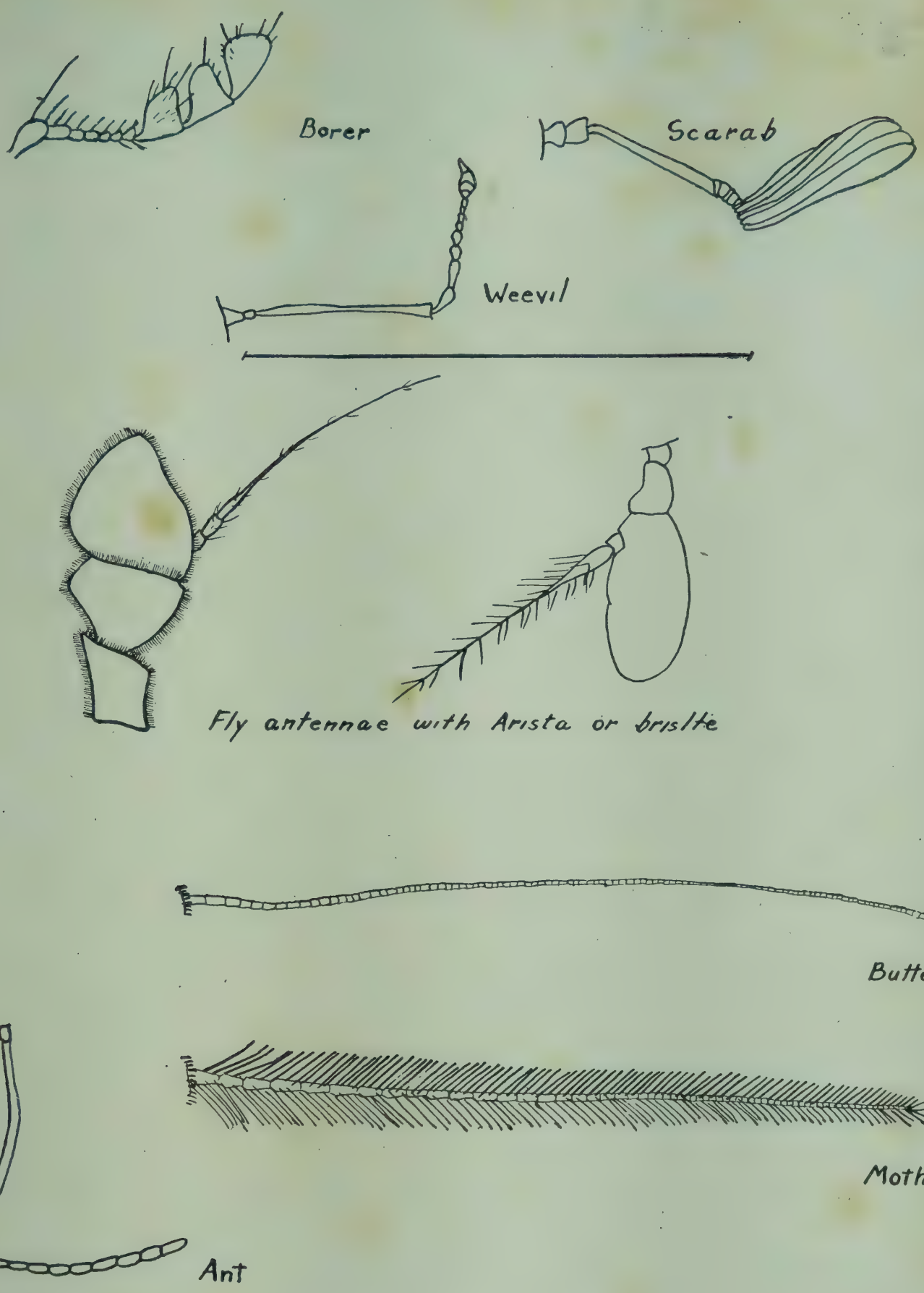


FIGURE 48.—Types of antennae.

or box of most of the insect orders. It covers the whole thorax and loosely used, it is this structure which, when the insect is viewed from above, commonly is called the thorax. In caterpillars and in beetle larvae its homologous structure is the pigmented prothoracic shield. The wings are fastened to the dorso-lateral aspect of their respective thoracic segments by a rather complicated articular structure at the wing base which is associated with the leg articulations. The wings have no muscles within themselves and are moved by a pulling and

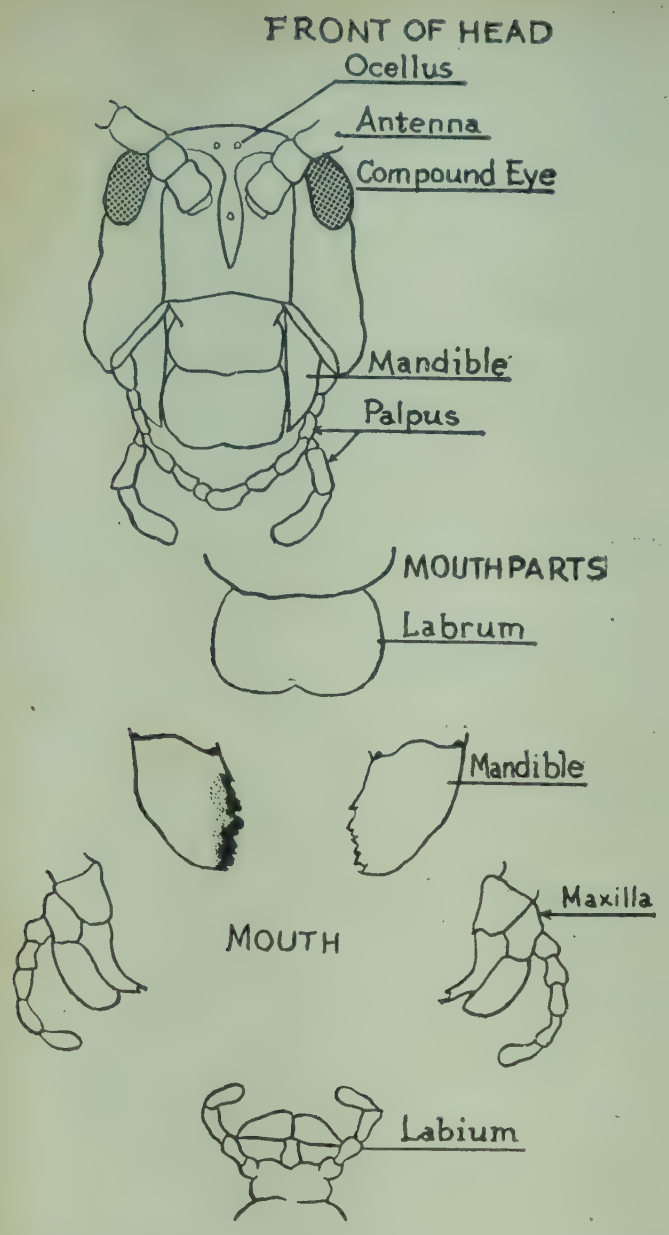


FIGURE 49.—Adult insect; head, and appendages.

elongation of each thoracic segment along the dorso-ventral and anterior-posterior line. Both legs and wings are joined to the thorax by a membranous basal area. The leg musculature extends from the cavity of the thorax into the last tarsal segment.

The fore wings are membranous, horny and leathery. The hind wings are membranous (Slides I–15, I–20, I–R–20). Either pair may be naked or hairy or scaly, pigmented or clear, many-veined or few-veined. Moth and butterfly wings are examples of membranous wings densely clothed with scales. Some fly wings are spotted with a thin covering of scales.

Larvae and nymphs do not have functional wings. The wings are characteristic of the adult form. The larval thoracic feet are the forerunners of the adult legs. They usually are cone-shaped, fastened to the body at the base of the cone, the apex terminating in a single hooked claw. The joints are distinct or indistinct and may appear only as wrinkles in the skin.

The adult leg has five parts: Coxa, trochanter, femur, tibia, and tarsus (Figure 51). The coxa may be only a short wedge-shaped segment that appears to be part of either the thorax or the trochanter. The trochanter is the first large-sized segment. Quite often it is round and stubby. The femur and tibia are elongate—these are the two elongate segments of the grasshopper's leg—and are the most prominent portions of the legs. The tarsus is the "foot." It may contain one to many joints and terminates in one or more straight or curved claws or a prehensile pad or both.

3. The Abdomen.

The union of the thorax and abdomen in the adult may be broad or constricted. Often there is a narrow petiole, the shape of which is characteristic of a particular group or even a particular species. In ants, for example, the petiole is several-jointed, each joint of a particular humped shape so that a person familiar with the ants might be able to recognize the species just from the petiole. If the petiole is short, the thorax and abdomen appear to be fused along a wide suture. The larval thorax-abdomen line is similar to that of any other suture and together the slender, elongate thorax and abdomen give the larva its worm-like appearance.

As is the case with the rest of the larva, body hairs, spines, or setae may be present and often form a characteristic-specific pattern. Each segment of both body regions typically has a pair of laterally placed spiracles which are the openings to the breathing tubes. The caterpillars have four to six pairs of pseudopods (see Figure 65 on caterpillar parts) on the ventral side of some of the abdominal segments, there often being a terminal pair on about the last segment, termed the anal pseudopods. These pseudopods function as feet, but they are unjointed and have no analogous structure in the adult. They have a sucker-like appearance. The distal end is formed like an open cup

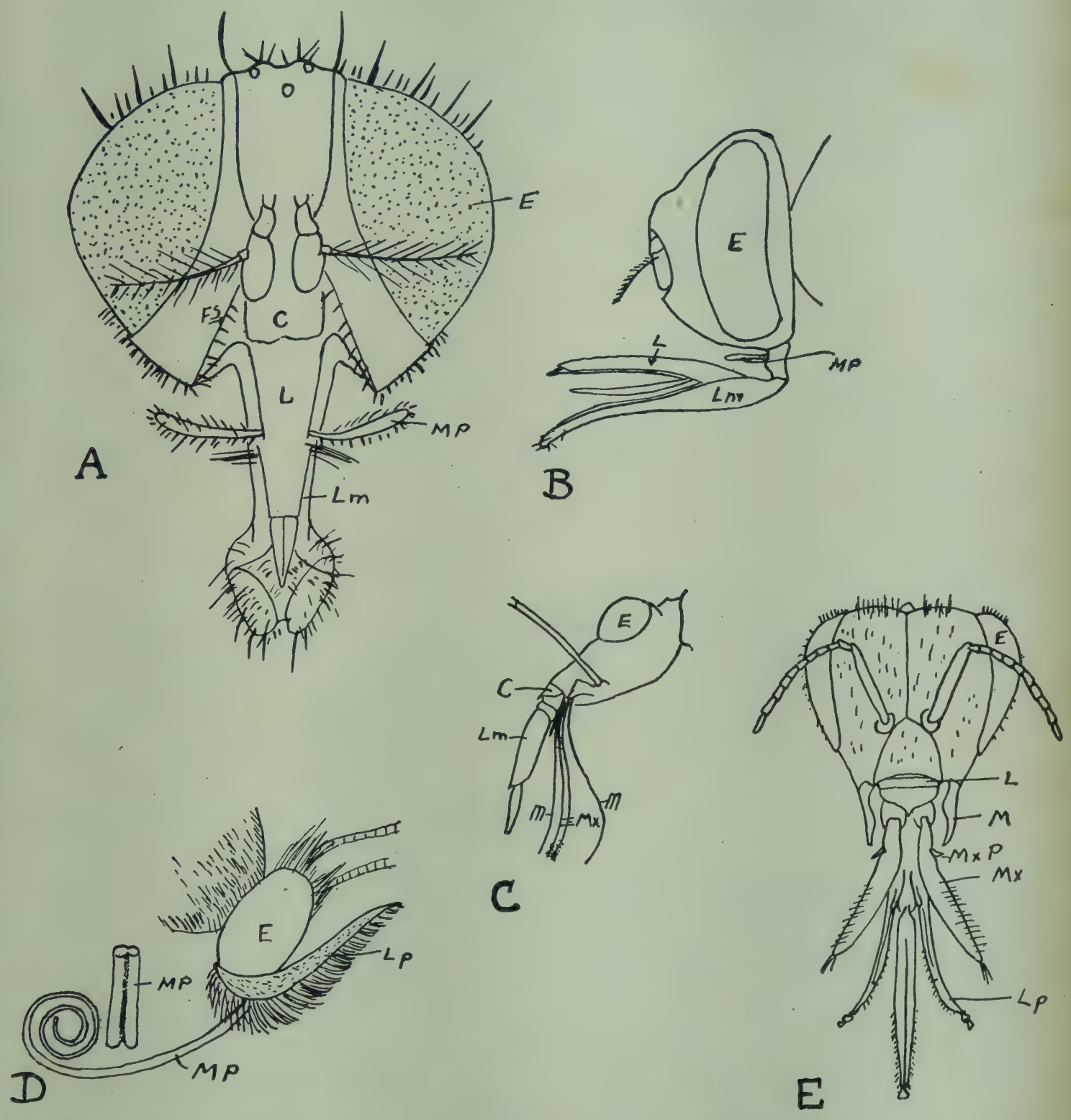


FIGURE 50.—Insect mouth parts. *A,* House fly; *B,* Stable fly; *C,* Hemiptera (teased apart); *D,* Moth ("tongue" in section); *E,* Honey bee (teased apart). (After Herms, Medical and Veterinary Entomology, ed. 2. By permission of The Macmillan Co.)

Key: C = clypeus; E = compound eye; FS = frontal suture; L = labrum; Lm = labium; LP = labial palpus; M = mandibles; MP = maxillary palpus; Mx = Maxillae.

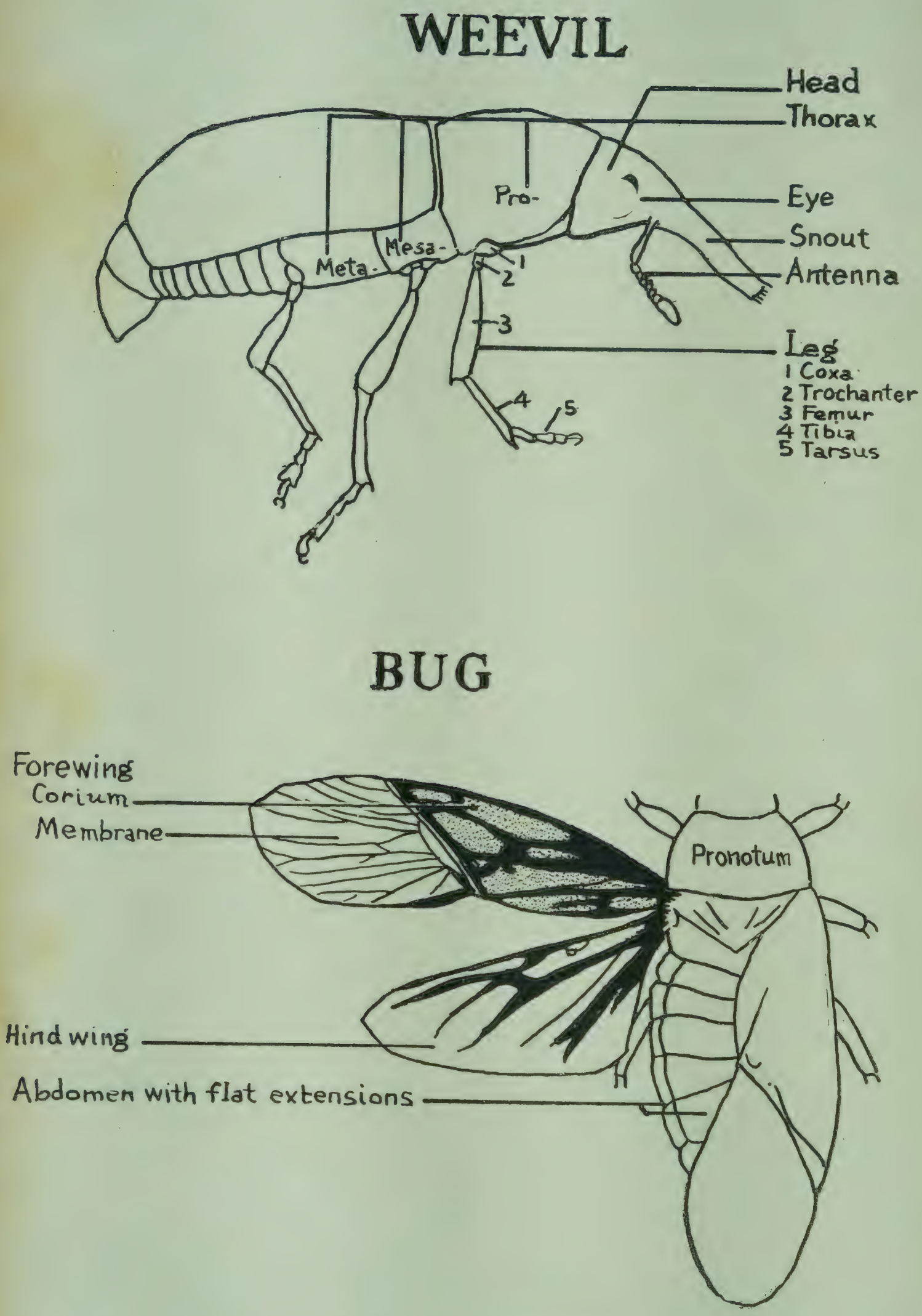


FIGURE 51.—Adult insect parts.

with a row of hooks projecting inward from the edge. Usually there are around 6 to 10 segments in both the larval and adult abdomen, there being a tendency to a reduction in number from the maximum of twelve. Ornamentation is either present or absent—hornlike structures being commonly found at the posterior end.

The abdomen simply is a sack full of viscera. The segmentation is distinct (coleoptera), indistinct (certain homoptera), or absent (other homoptera). Usually there is a tapering posteriorly and the organs of

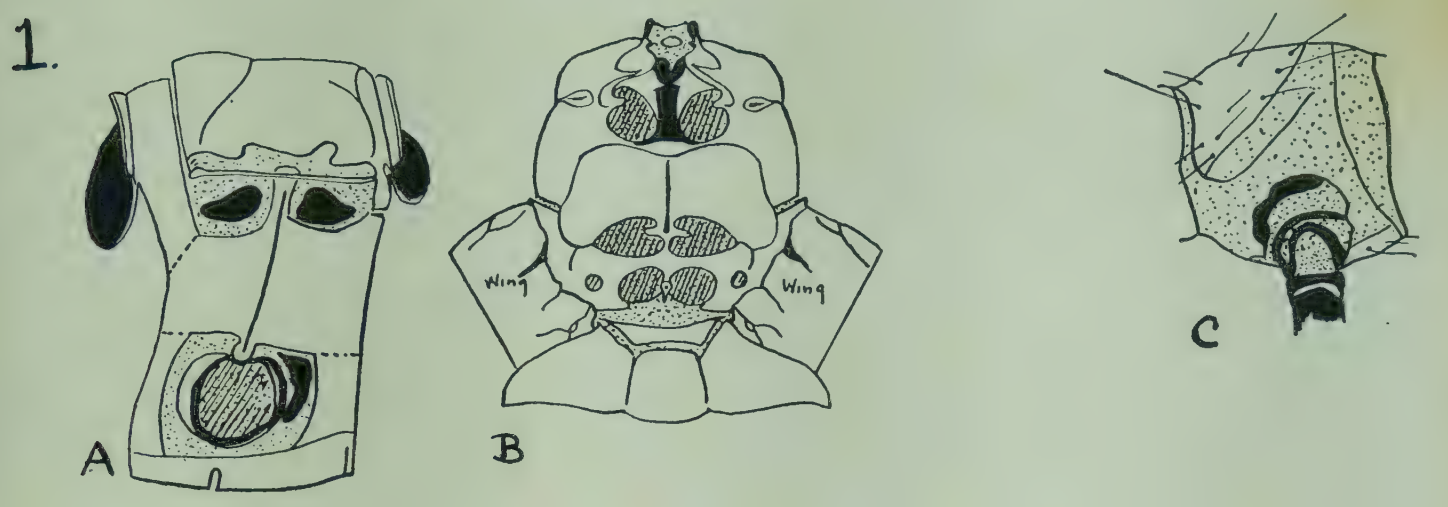


FIGURE 52.—1. Various generalized thoracic views giving a rough picture of the sclerites (black or white = sclerites, stippling = membranes, cross-hatch stipple = leg cavity). *A*, Lateral view, and *B*, Ventral view of wing-bearing segments; *C*, Lateral view of apterous segment. 2. Some details of leg structure. *A*, *B*, *C*, Articulations; *D*, *E*, *F*, "Foot structures"; *G*, Larval "foot." (From Snodgrass, Principles of Insect Morphology. By permission of McGraw-Hill Book Co.)

copulation occur usually at the extreme posterior end. These structures are very important taxonomically but a study of their structure must be left to the work of specialists and is unsuited to a summary such as this.

Of the internal structures, the only ones of which have been of importance in insect fragment identification work are the mouthhooks and trachea. The mouthhooks were figured earlier. The trachea, branching into the tracheoles are the breathing tubes of insects. Air taken in through the spiracles is distributed throughout the body through this system of tubes. The tracheal trunks anastamose one with the other and then branch and rebranch becoming finer and finer. The cuticle of the larger trunks is a membrane thickened with spiral filaments.

B. Insect Life Histories

The foregoing has been a generalized description of commonly encountered insect adults and larvae. It will be remembered that some groups have nymphal forms and no larvae. In these the young, or nymphs, resemble the adults but are smaller, often more pale, and

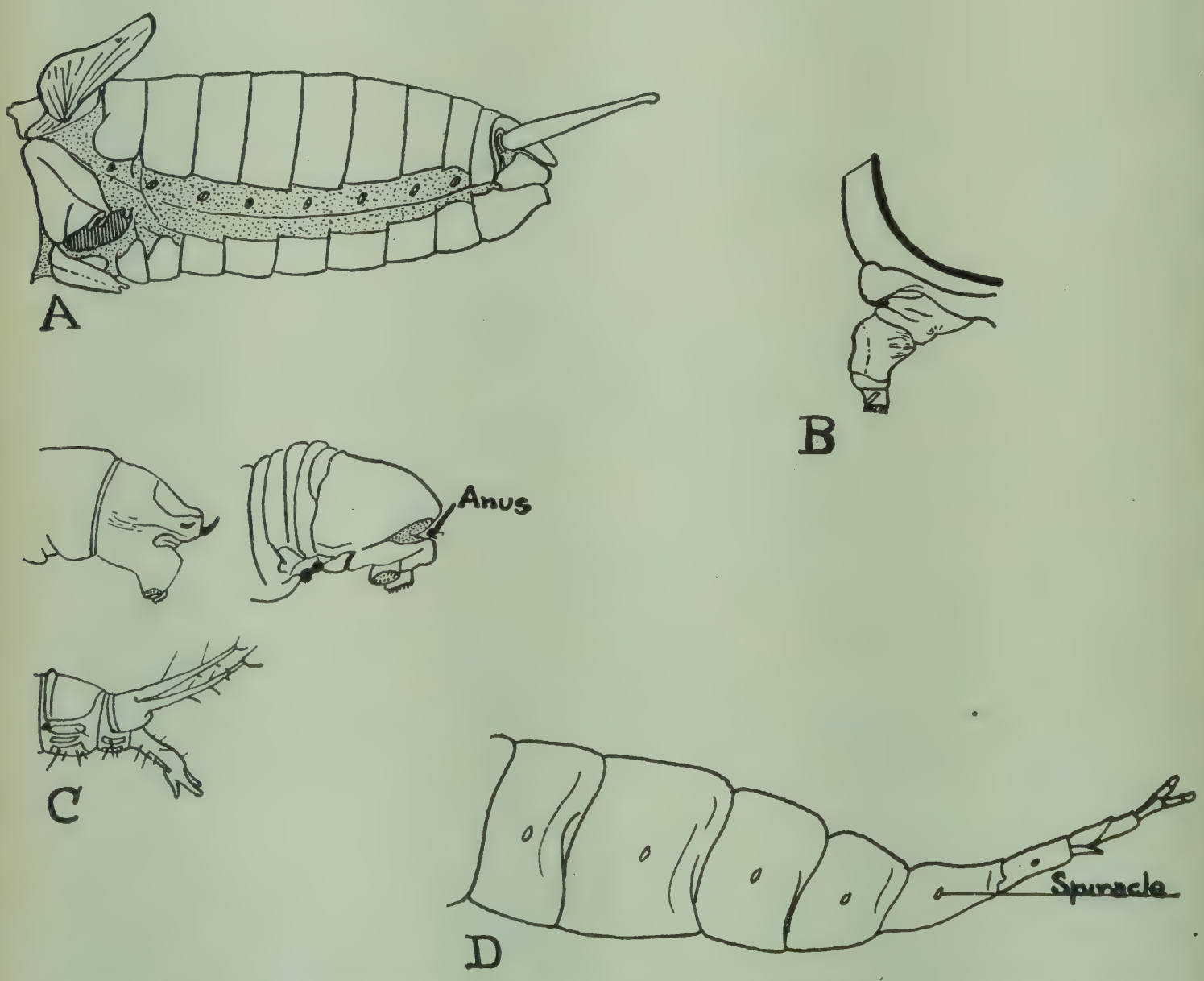


Figure 53.—The abdomen. *A,* Lateral view showing rows of dorsal and ventral plates, lateral membrane with row of spiracles and plates; *B,* Abdominal pseudopod, larva; *C,* Terminal abdominal appendages, larva; *D,* A type of fly abdomen. (*A, B, C,* after Snodgrass, Principles of Insect Morphology. By permission of McGraw-Hill Book Co.)

Weevil

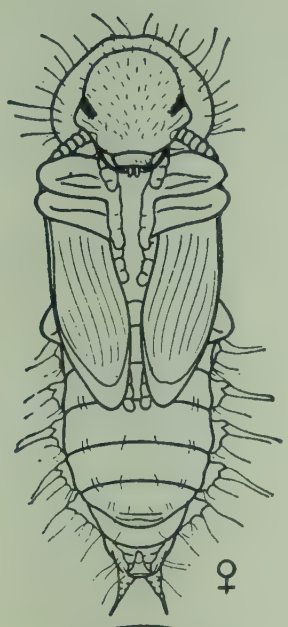
Borer

Flour Beetle

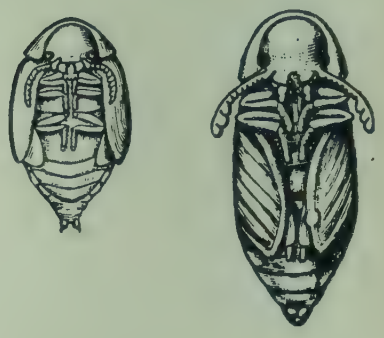
Grain Beetle

Granary Weevil

Saw-toothed Grain Beetle



Tribolium sp.



Drug Store Beetles

Bean Weevil

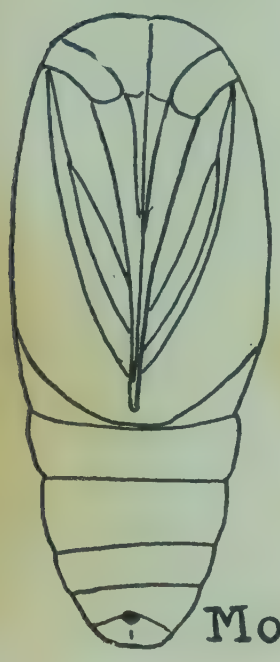
Mealworm

FIGURE 54.—Beetle pupae. (From Farmers' Bull. 1260 and 1275, U. S. Dept. Agric.)

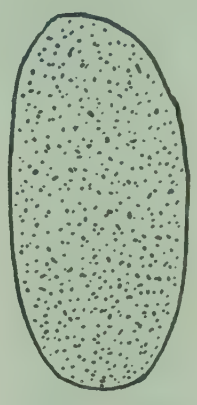
none have functional wings although wing pads may be present. In many groups there is a stage intermediate between the larva and adult —the pupa.

The pupa appears as a folded-up adult. All of the adult structures,—mouth-parts, legs, and wings,—show through a tightly fitting cover as they are folded against the sides of the developing adult. Comminuted in foods the pupae contribute many perplexing fragments. Large portions of their outer covering are almost devoid of any landmarks except an occasional suture. In addition, many pupa coverings are pale tan in color and closely resemble in color both bran, chaff, and peeled-off enamel lining from the interior of a can.

Most insects hatch from eggs (are oviparous) although some are larviparous (some diptera) and others give birth to living nymphs (e.g. some homoptera) (Figure 56 *B*). Insect eggs are oval to round, usually somewhat elongate, and sometimes noticeably kidney-shaped. They are laid singly or in clusters; the covering may be smooth, irregularly rough, or with a pattern, spines, plumes, or other ornamentation.



Moth

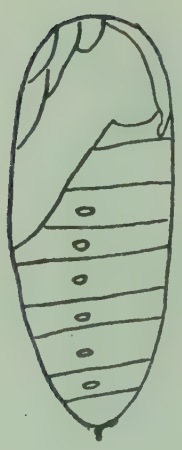


Fly Puparium and Pupa

Tomato Pinworm

Corn Borer

Hornworm



Sphinx Moth

Wasp

Fly

Ant

FIGURE 55.—Pupae. (Wasp from Bur. Ent. Bull. 85, U. S. Dept. Agric.; fly from Illinois Agric. Expt. Sta. Circ. 437; ant from Leaflet 147, U. S. Dept. Agric.)

Depending upon the habits of the species, they may be deposited in, on, or near food and allowed to lie loose and exposed or glued down with a matrix which hardens. Some eggs are laid several to many together in a covering (e.g. orthoptera oothecae).

The length of the time from egg through a life cycle to egg again will vary enormously. Under optimum conditions the lower limit may be around 3 weeks and the upper limit is probably that of the 17-year locust which takes 17 years. An insect which may have a life cycle of 4 weeks may be caught in a cold spell or over winter to begin the next spring where it left off months before. All the various environmental factors, as well as the characteristics of the species, are important. However, during the summer most field pests spend about a month getting from egg to adult and as a general rule the various storage beetles and moths take about the same length of time.

FIGURE 56.—Metamorphosis. *A,* Complex or complete: Egg, larva *a,* pupa *b,* adult *c* (fly, left, and bean weevil, right); *B,* Simple: Egg, several nymph stages, *b, c, d;* adult *a* (thrips, left, and aphis, right). (From: *A,* fly and *B,* thrips, Illinois Agric. Expt. Sta. Circ. 437; *A,* bean weevil, Farmers' Bull. 1275, *B,* aphis, Farmers' Bull. 1371, U. S. Dept. Agric.)

If the life history takes about a month, then the egg stage may take 3 to 5 days, and 3 weeks is taken up as a larva and pupa, the pupal period lasting longer than does the egg. The so-called "lower" insects hatch from the egg resembling the adult, except that they are smaller and have no full wings, although rudimentary wing pads may be present. In the accompanying discussion of insect groups, all of the groups up to and including the hemiptera are of this type. The immature forms are called nymphs. The nymph which hatches from the egg will cast its outer skin after a day or more. This process of casting off the old skin is known as molting, and is necessary because the tough insect exoskeleton will not stretch, and in order to grow the insect forms a new skin beneath the old one, casts off the old, swells up and hardens the new skin, and then proceeds to grow into it. This skin, in turn, will be discarded, and so on until maturity. The winged insect is always an adult, although many species of adults do not have wings. The coleoptera and groups phylogenetically above them all go through a larval and pupal stage. A wormlike larva hatches from the egg. This larva grows and molts its skin until after around five to seven molts it changes into a pupa. Some pupae use the last larval skin for a pupa case. Others form a new case which resembles a very much folded-up adult. This is especially pronounced in the beetles. Many moths are noted for spinning a cocoon of silk around the pupa or chrysalis. Within the puparium the adult develops and it in turn splits off the outer covering and emerges.

If the insect is living in food, the casts will often be found when a subsequent examination is made for the insects. Some insects eat their cast skins and if this is the case the fragmented skins are left in the food as part of the insect excreta.

C. The Insect Orders

The arachnids and insects are closely related in the phylum Arthropoda. Together they commonly are called insects or bugs, although scientifically the first term is reserved for six-legged arthropods and the second term for a particular order of insects. Considering their numbers, the spiders seldom are found in foods; if and when they do appear the contamination usually is not one that is caused by poor commercial practices. The mites often are troublesome pests on a variety of products both in the field and in storage. The insects themselves are present in and on foods. Almost all of the orders have representatives which contaminate food; many of them are serious and repulsive pests. Of the arthropods we are chiefly concerned with the following groups:

CRUSTACEA: Aquatic or inhabiting damp places; gill respiration; two pairs of antennae; biramous appendages; e.g., the sow bug.

MYRIAPODA: Body elongate, worm-like and segmented; each segment except first two and last one bearing one pair of jointed walking appendages (centipedes), or bearing two pairs (millipedes). (Figure 57).

ARACHNIDA: Usually 4 pairs of legs; wingless; no antennae; eyes simple when present. (Figure 58.)

ARANEIDA: Head and thorax fused into cephalothorax bearing 4 pairs of legs; two body regions, cephalothorax and abdomen, joined by a narrow stalk (spiders).

ACARIDA: Ticks and mites. Abdomen fused with cephalothorax into one body region.

(a) Mites, minute: Exoskeleton soft, four pairs of legs in the adult, distributed somewhat over entire ventral aspect. (b) Blister mites. Exceedingly small, elongate with two pairs legs at anterior end. Body consists of a short cephalothorax bearing legs, palpi, and rostrum, and of a faintly segmented elongate abdomen. (Figure 58 *B*, 60 *C*.)

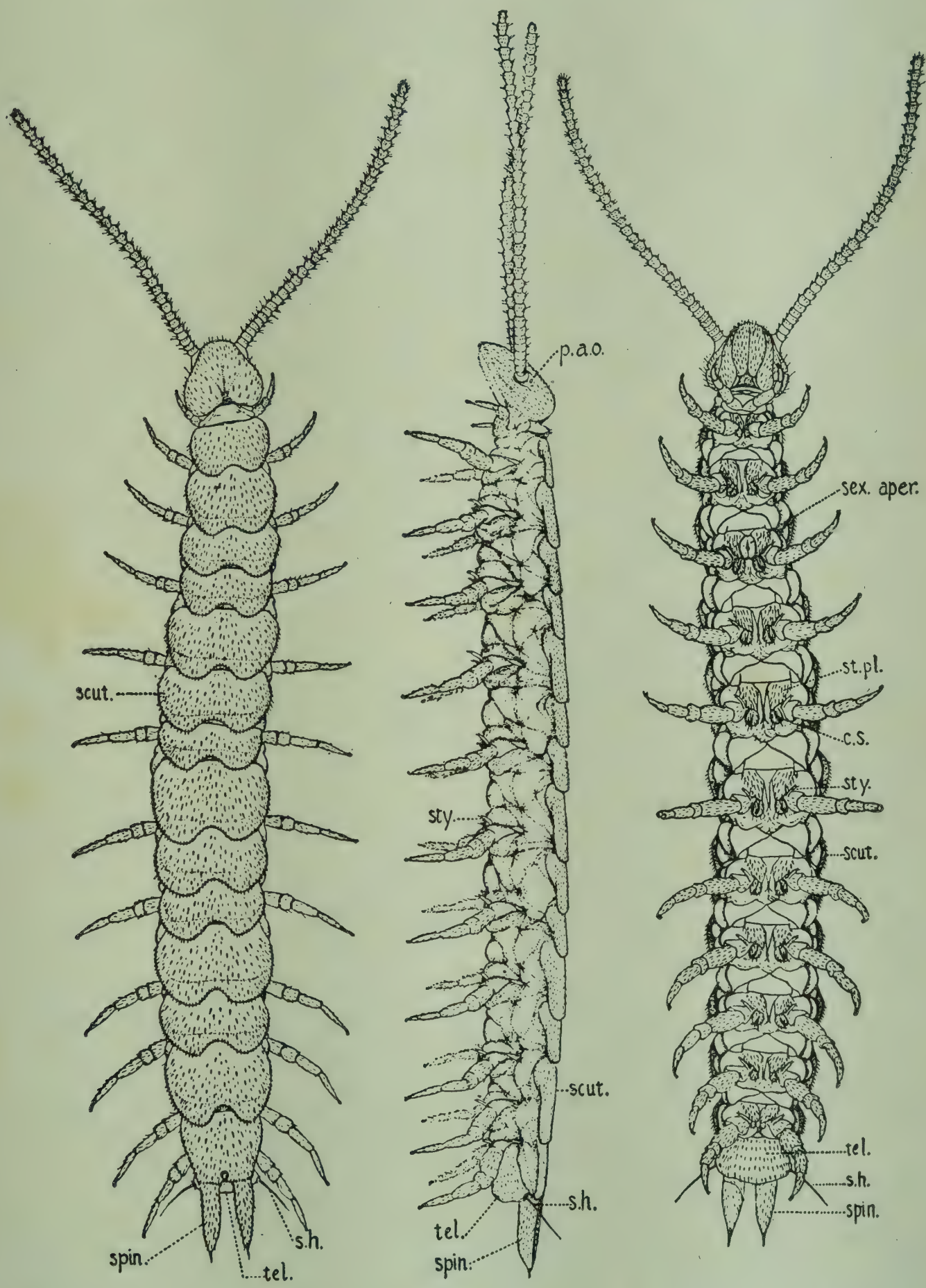


FIGURE 57.—Adult garden centipede. (From Hilgardia **11**: 55 (1938). Michelbacher, The Biology of the Garden Centipede.)

INSECTA: Adults with three pairs of legs; body divided into three regions: a head with mouth part, eyes, antennae, etc.; thorax bearing the legs, and wings when present; and a more or less segmented abdomen. Immature stages are either worm-like larvae, or nymphs or pupae or somewhat resembling the adult.

The following arrangement of orders is somewhat based on morphological relationships but mainly is arranged for convenience:

SILVERFISH, FISH MOTHS (THYSANURA). Somewhat flattened, long antennae, three long and several short anal appendages, body covered with grayish silvery scales and hairs, simple metamorphosis, i.e., no larval or pupal forms. The house-infesting forms are swift, nocturnal insects that feed upon starchy materials. They usually are not serious pests except around poorly kept bakeries and kitchens or under moist conditions. Reference: Silverfish, Leaflet 149, U. S. Dept. Agric.

SPRINGTAILS (COLLEMBOLA): Small delicate white or grey, long antennae and anal cerci, live in damp soil or damp or decaying vegetable matter. These insects are of minor importance as food-infesting pests except for those which infest mushrooms and may be present in or on mushrooms in large numbers. They work in the mushrooms while they are growing in the field and because of their tunneling and presence in the gills they remain in the dried product. The tunnels are distinctly visible in the fresh product and badly infested mushrooms are apt to be marketed dried. (Reference: Mushroom Insects, Penna. Expt. Sta. Bull. 419).

COCKROACHES (ORTHOPTERA): Medium to large, simple metamorphosis, bodies regularly oval and flat, pronotum projects over the head,

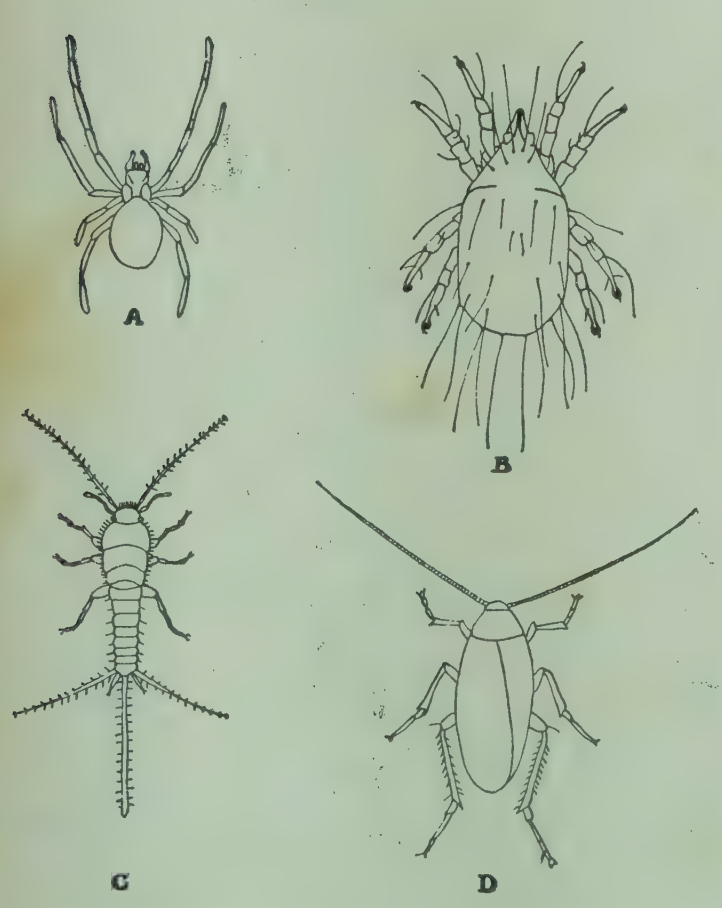


FIGURE 58.—Arachnid and insect orders. *A,* Spider; *B,* Cereal mite, 0.33 mm.; *C,* Silverfish; *D,* Cockroach. (*B* after Essig, Insects of Western North America. By permission of The Macmillan Co. *C* from U. S. Dept. Agric.)

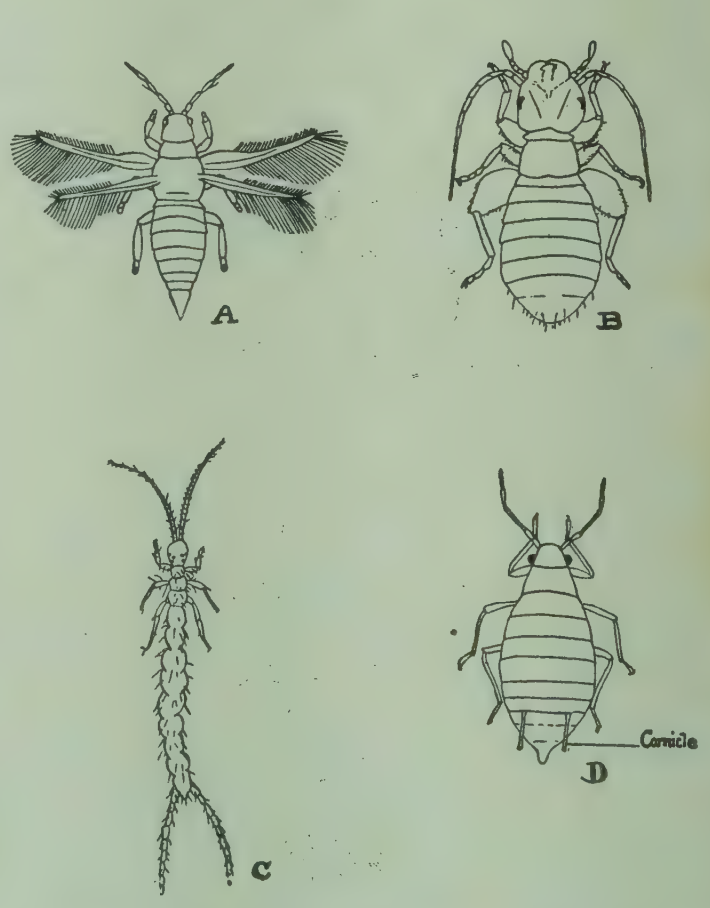


FIGURE 59.—Insect orders. *A,* Thrips, 1.2 mm.; *B,* Cereal psocid, 1 mm.; *C,* Campodeid, 3 mm.; *D,* Wingless aphis, 2–3 mm. (*C* after Essig, Insects of North America. By permission of The Macmillan Co.)

long slender antennae, leathery front wings and membranous hind wings, eggs laid in peculiar masses called ootheca. They are swift runners, nocturnal in habit, feeding upon a variety of foods indoors or vegetable matter in the field. Under filthy conditions they may literally swarm forth at night from their hiding places such as cracks, behind cabinets, etc. In some places where all of the surrounding area is infested with roaches a few may wander into even a well-kept establishment, but with care it is possible to keep their numbers down to a relatively few. Along with rats and mice, cockroaches are among the most disgusting pests that may infest human dwellings. (Reference: Cockroaches and Their Control, Leaflet 144, U. S. Dept. Agric.)

GRASSHOPPERS AND CRICKETS (ORTHOPTERA). Accidentally may get into foods and occasionally a large piece of one is found but they do not represent any particular trouble from a regulatory standpoint.

PSOCIDS (CORRODENTIA) (Figure 59). Small to minute insects with simple metamorphosis and biting mouth-parts (food-infesting forms usually wingless). The body is somewhat flattened. A few species inhabit buildings and feed on cereals. At times they may occur in large numbers especially when the food or warehouse is damp. In these instances they may be swarming over the food, floors, cracks and crevices, and between the walls. Even when the food is removed they may continue to appear in considerable numbers. Reference: Psocids, Leaflet 189, U. S. Dept. Agric.

THRIPS (SINGULAR THRIPS) (THYSANOPTERA) (Figure 59). Small slender insects; metamorphosis simple; the cone-shaped, piercing and sucking mouth-parts are situated far back on the ventral side almost at the front legs. Usually there are two pairs of long narrow wings (not in the nymphs which otherwise resemble the adults) with few or no veins and an outer marginal row of long hairs. Antennae are often composed of barrel-like segments. There are many species of thrips feeding on the leaves and fruits of various plants and holding on tightly or crawling down in cracks, between berry drupelets, etc., so that they may persist in the prepared food, especially when the leaf or green pod is used, as in spinach or green beans, or when the fruit is eaten unpeeled. Reference: Insects of Western North America, E. O. Essig, page 179.

LEAFHOPPERS, SPITTLEBUGS (HOMOPTERA). Small insects, broadly rounded in front and tapering gradually posteriorly. The nymphs often live in a mass of white froth or spittle. The antennae are minute and bristle-like; mouth-parts are adapted for sucking. Occasionally one that has accidentally flown into a batch of food may be encountered in the finished product, but they are not food-infesting.

TREEHOPPERS (HOMOPTERA). Queer-looking small insects characterized by the enlarged and prolonged prothorax which projects above the head and extends back over the abdomen to make the insect look like a spine. They are not food-infesting.

APHIDS (HOMOPTERA). Small to minute; delicate; plump-bodied; winged or wingless; sucking mouth-parts; when present, fore and hind wings of approximately the same size and texture; antennae well developed; rostrum arises well back on the underside of the head. There is scarcely any type of vegetation which does not have its aphid pests

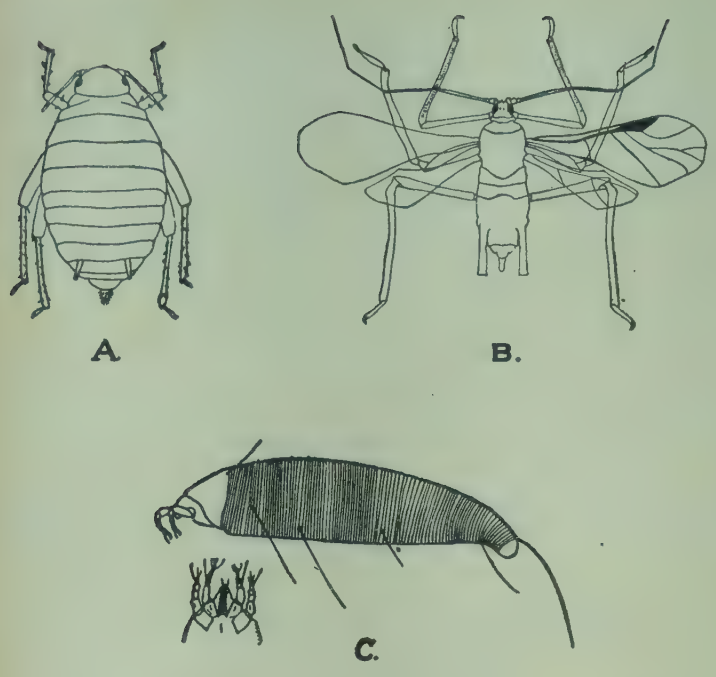


FIGURE 60.—Insect orders. *A,* Wingless aphis; *B,* Winged aphis; *C,* Blackberry mite. (*C* after Oregon Agric. Expt. Sta. Bull. 337.)

(Figure 60). They may feed on leaves, stems, roots, and fruits. Metamorphosis simple. The egg stage may be omitted entirely and enormous numbers of offspring may result from a few parthenogenetic females. On plants with rough surfaces, overlapping leaves, or flower clusters (such as mustard greens, brussels sprouts, broccoli, etc.), it is difficult to remove the aphids commercially once they are present. This group contains many prolific pests. Reference: The Turnip Aphid. . . , Farmers' Bull. 1863, U. S. Dept. Agric.

SCALE INSECTS (HOMOPTERA). On processed foods only the stationary forms (covered with a waxy secretion or shell) are usually found. The winged migratory forms are seldom seen. The stationary insect has degenerated into an exceedingly minute form with practically no eyes, rudimentary antenna, and simple legs terminating in a single claw (Figure 61). The rostrum, containing piercing and sucking stylets, arises far back on the head. Scale insects may be present in enormous numbers although in certain foods (citrus peel, ground spices, etc.) the infested portion usually is discarded and the insect infestation presents no great difficulty from the food-contamination standpoint. Reference: The External Anatomy of the Red Date Scale, Tech. Bull. 404, U. S. Dept. Agric.

BUGS (HOMOPTERA). Insects distinguished by the half-leathery, half membranous fore wings; hind pair are membranous; at rest, wings folded on back form an "X"; mouth-parts for biting, piercing, and sucking; young resemble the adults. This group of easily distinguished insects contains many important plant pests but they are rather unusual

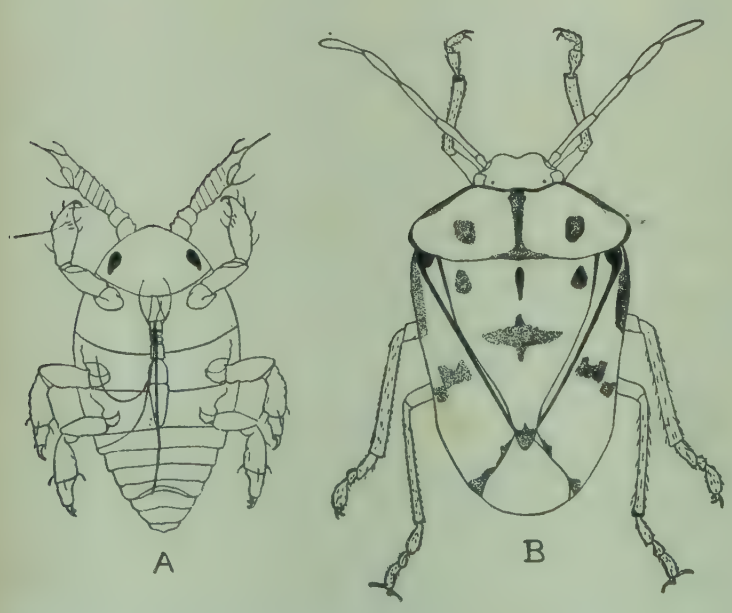


FIGURE 61.—Insect orders. *A,* Ventral aspect scale insect; *B,* Cabbage bug.

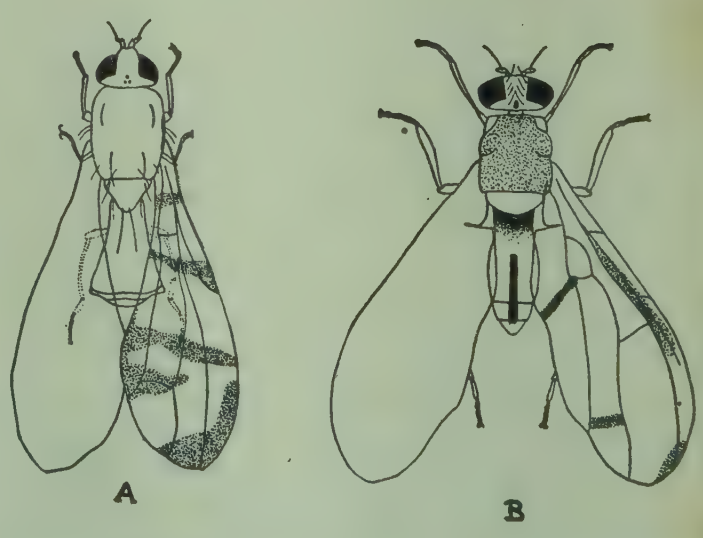


FIGURE 62.—Insect orders. *A,* Gooseberry fly; *B,* Melon fly.

as food contaminants, although occasionally a whole bug is found in a pack of vegetables such as greens or green beans. They are active plant feeders or are predaceous on other insects and they do not burrow into foods or hide in the foliage.

BEETLES AND WEEVILS (COLEOPTERA). This is a very large order of conspicuous insects with horny exoskeletons, horny or leathery front wings (elytra), strong biting and chewing mouth-parts, and complete metamorphosis (egg, larva, pupa, adult). Most of the larvae are worm-like with or without three pairs of legs, and with biting and chewing mouth-parts. Both adults and larvae are serious pests of growing plants and stored foods. (See "Key to the Common Storage Insects," p. 97.)

FLIES AND MAGGOTS (DIPTERA): (Slides I–R–17, I–R–23). The flies also constitute a large order of insects (Figure 62). They have one pair of wings the hind pair being represented by two knobbed halters, (Slide I–21) mouth-parts are for biting, piercing, sucking, or lapping; head united to the thorax by a slender neck; two large compound eyes usually take up the greater part of the head, and there usually are three ocelli. Metamorphosis is complete. The legless larvae are called maggots; they are eyeless; paired pseudopods may be present or absent; there may be ventral bristles that aid in movement, but a distinct horny head covering and, in fact, a rigid exoskeleton usually is absent. (Figure 63.) Maggots usually live in aquatic or damp locations so that both flies and maggots often frequent filth, garbage, decaying animal and vegetable matter, etc. Flies are among the filthiest of insects, some species living intermittently on excrement and human food. Because of their fondness for decaying matter, some species of flies can be used as an index of filth or decomposition. In addition, many flies are hairy and have sticky mouth-parts and feet which are admirably adapted for the carrying of micro-organisms. The case against the housefly traveling between fecal matter and human food has been widely advertised but other contaminations are less widely appreciated. This same housefly can carry disease-producing micro-organisms from rat excreta to food. Maggots can live in unprotected batches of sauerkraut, rotting or overripe fruits and vegetables, pickling vats, on any moist food, meat, etc. Some maggots infest growing plants in the field. In this case they usually are found within the stem, leaf, root, or flower so that it is more difficult to find them than is the case with many surface feeders. The spinach leaf miner and asparagus miner are of this type. Other forms infest soft fruits and berries. Gooseberries, blueberries, cherries, etc., all have their maggot pests.

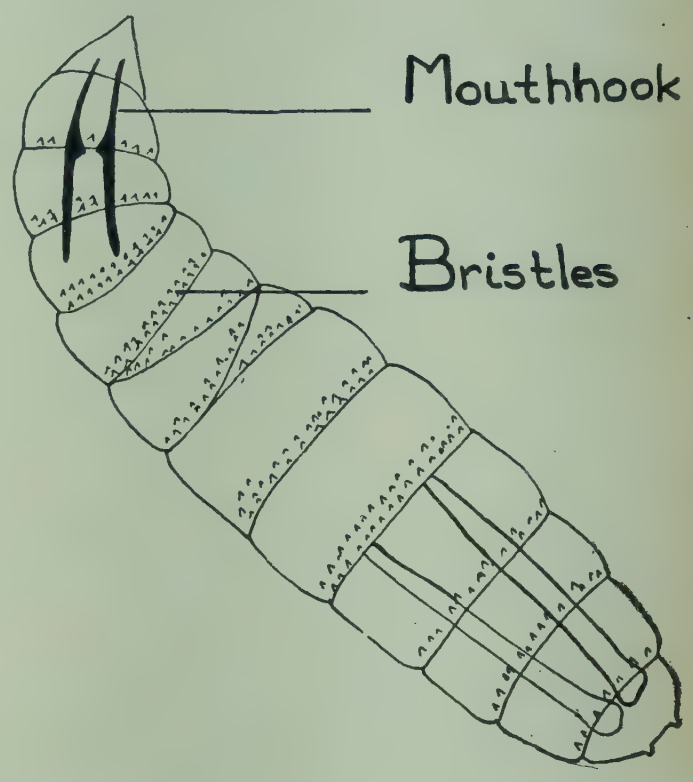


FIGURE 63.—Insect orders. Maggot. (After Leaflet 162, U. S. Dept. Agric.)

References: Housefly Control, Leaflet 182, U. S. Dept. Agric.; The Biology and Control of the Blueberry Maggot in Washington County, Maine, Tech. Bull. 275, U. S. Dept. Agric.

BUTTERFLIES, MOTHS, CATERPILLARS (LEPIDOPTERA). Adults usually have four well-developed wings entirely or partially covered with overlapping scales; mouth-parts suctorial or abortive. Larvae, called caterpillars (Figure 65), are wormlike, with three pairs of thoracic or true legs and often several pairs of sucker-feet or pseudopods, and with biting and chewing mouth-parts. Many of our most common and destructive plant pests belong to this group. (Figure 64). The adults usually are rather strong fliers. They themselves do no damage other than the laying of eggs but the larvae get into practically all our fruits, vegetables, cereals, etc. They work in or on the fruit, leaf, and on the growing plant or in storage. The following insects are among the most notoriously famous caterpillars: The cabbage worm, *Pieris rapae;* the

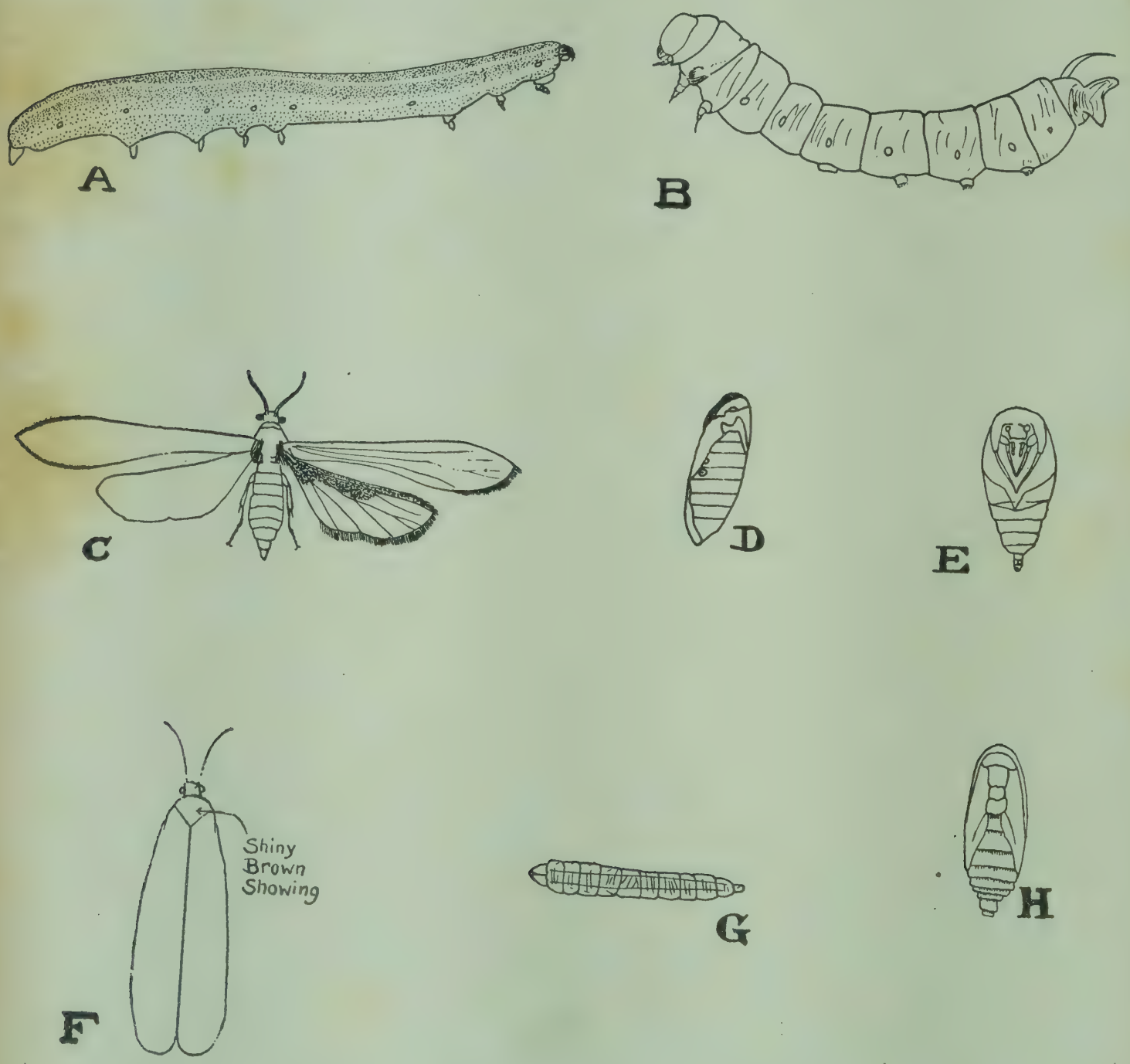


FIGURE 64.—Insect orders. *A,* Army worm; *B,* Hornworm; *C,* Adult peach borer; *D,* Army worm pupa; *E,* Tent caterpillar pupa; *F,* Adult codling moth; *G,* Peach borer; *H,* Codling moth pupa.

tomato worm, *Protoparce sexta;* corn earworm (tomato fruitworm), *Heliothis obsoleta,* the beemoth or waxmoth, *Galleria mellonella;* Mediterranean flour moth, *Ephestia kuehniella.* References: Caterpillars Attacking Tomatoes, Univ. of California, College of Agric. Bull. 625; The Angoumois Grain Moth, Farmers' Bull. 1156, U. S. Dept. Agric., The Cannibalistic Habits of the Corn Ear Worm, Tech. Bull. 499, U. S. Dept. Agric.; The Wax Moth and Its Control, Circular 386, U. S. Dept. Agric. See also Key to Storage Insects.

FIGURE 65.—Caterpillar parts. (From California Agric. Ext. Serv. Circ. 99.)

BEES, WASPS, ANTS, PARASITES (HYMENOPTERA). This order, as a whole, is highly variable. Mouth-parts are biting, lapping, or sucking. They have two pairs of membranous wings, abdomen joined to the thorax by a narrow petiole which in some groups (e.g., ants) (Figure 66*A*) is highly characteristic; larvae, caterpillar-like, maggot-like, or very degenerate. Most of the hymenoptera are beneficial and are usually not found as contaminants in food. Honeybees, of course, are the source of honey and some of their parts are found in this product. Parasites of food-infesting species sometimes are found in food along with the pest and the fig blastophaga occurs in some figs. Most of the ants are wingless during most of their life and many species are troublesome food contaminants. Reference: House Ants, Leaflet 147, U. S. Dept. Agric.

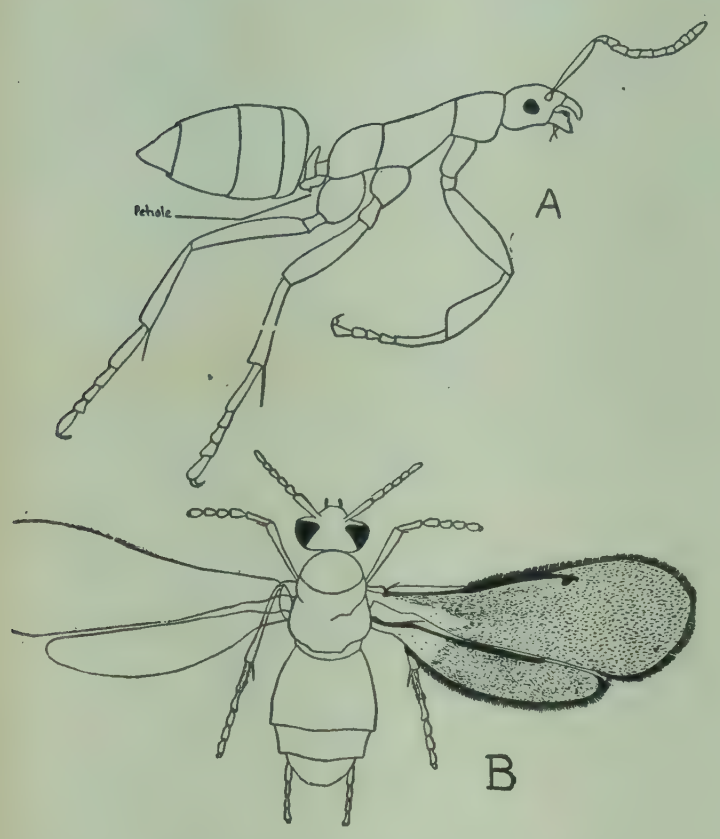


FIGURE 66.—Insect orders. *A*, Ant; *B*, Parasitic hymenoptera.

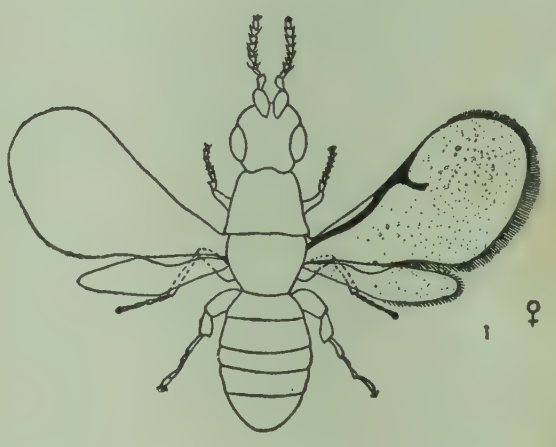


FIGURE 67.—Blastophaga fig pollenator

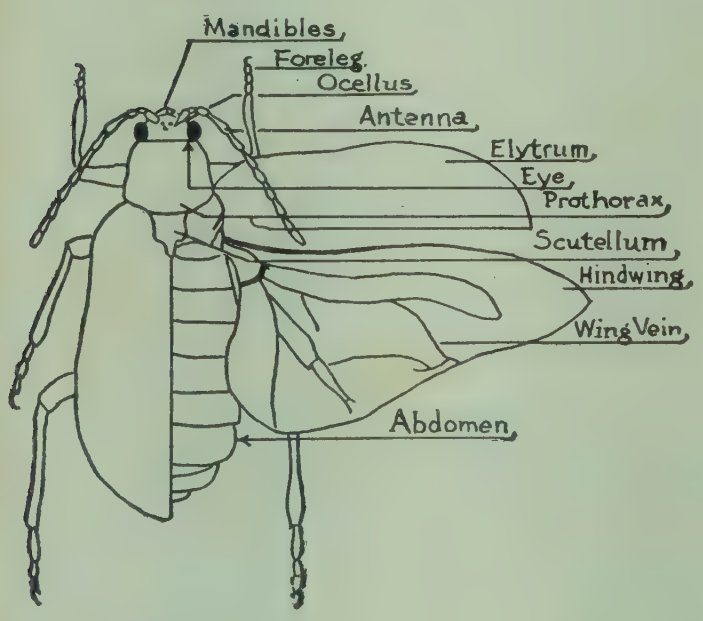


FIGURE 68.—Adult insect beetle, diagrammatic.

D. Key to the Common Storage Insects

A large proportion of the Administration's insect filth work concerns storage pests of various food products. To a large extent there are relatively few pests which have established themselves in man's food as he has spread over the earth. To be sure, each region has some unique forms which are not found elsewhere but whether we are dealing with grain from Australia, spices from the East Indies, chocolate from South America, or peanuts from Virginia the storage pests are about the same. Spice may also be infested with some pest of the growing plant but to classify such an insect beyond the order or group to which it belongs is far beyond the scope of this work.

This key, expected to cover most of the storage pests, is based upon a table in Minnesota Agric. Expt. Sta. Bull. 198, Insects Infesting Stored Food Products. From it the analyst may determine the species concerned or whether or not he has a pest of stored food.

I. Adults with hard exoskeletons; wings meet in straight line down the back. Larvae with three pairs of legs, if any. BEETLES, COLEOPTERA (Figure 69).

A. Adults with long snouts; antennae with "elbow" separating long segment from smaller segments. Larvae legless grubs (Figure 69, 1) living inside of solid material. WEEVILS.

1. Snouts narrow elongated. Adults slightly over $\frac{1}{8}''$ long; found in wheat, barley, corn, macaroni, or other hard products.

a. Adults brick red to blackish; pronotum covered with pattern of pits in uneven rows; elytra with four more or less obscure white areas. RICE WEEVIL, *Sitophilus oryzae*. (Figure 69, 1) (Slide I–R–4)

b. Adults brick red to blackish; pronotum covered with pattern of elongated pits; elytra unspotted. GRANARY WEEVIL, *S. granaria*. (Figure 69, 1) (Slide I–R–8)

2. Snouts broad. Adults slightly less than $\frac{1}{8}''$ long; attacks soft or damaged grain. BROADNOSED GRAIN WEEVIL, *Caulophilus latinasus*.

B. Adults without long snouts.

1. Adults reddish brown.

a. Adults half as broad as long; rather stout beetles.

(A) Adults hairy with minute lines down the back. Larvae with a few short hairs. DRUGSTORE BEETLES, *Sitodrepa panicea*. (Figure 69, 3) (Slide I–R–26)

(B) Adults hairy; no lines on the back. Larvae covered with hairs. CIGARETTE BEETLE, *Lasioderma serricorne*. (Figure 71, 2) (Slide I–R–19)

(C) Adults; long legs and antennae give a spider-like appearance. Larvae often found in small cases which they have

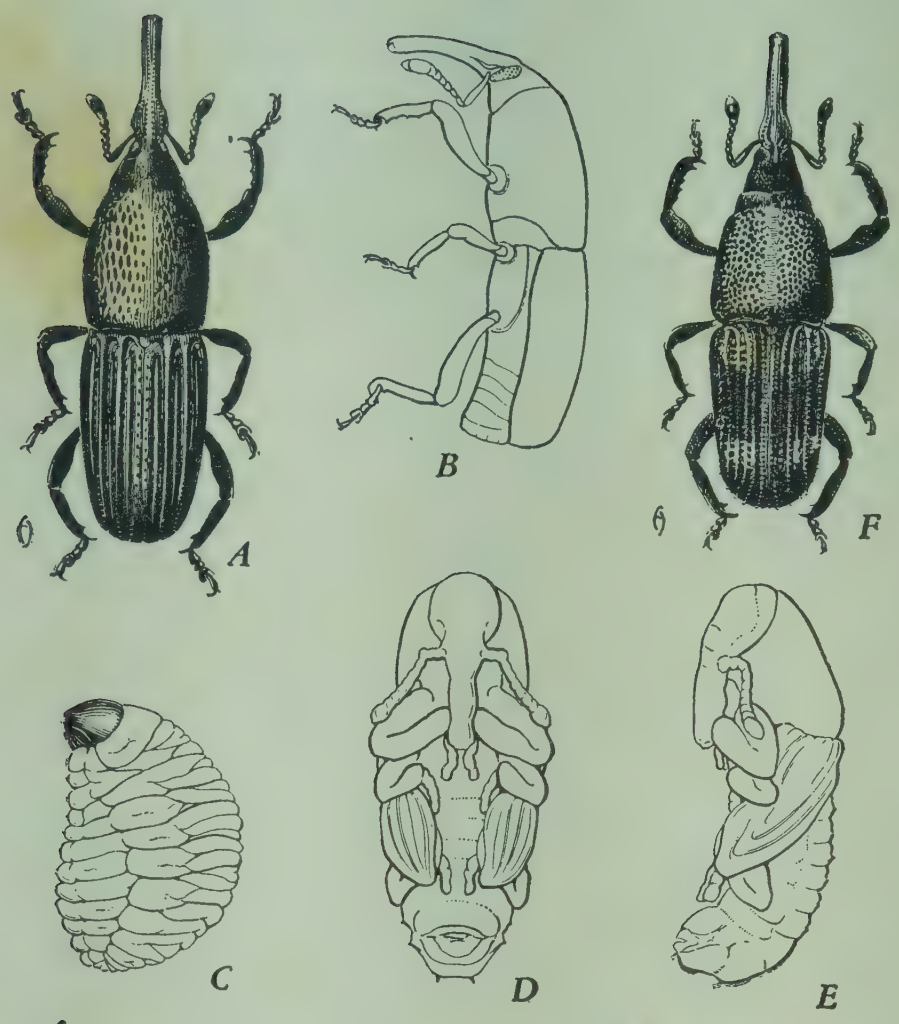


FIGURE 69.—1. *A–E,* The granary weevil; *A,* Adult; *B,* Lateral view adult; *C,* Larva; *D–E,* Pupa; *F,* Adult rice weevil. 2. Broadnosed grain weevil. 3. Drugstore beetle. 4. Hairy spider beetle. (From: 1—California Agric. Expt. Sta. Bull. 676; 2, 4—Farmers' Bull. 1260, U. S. Dept. Agric.; 3—Minnesota Agric. Expt. Sta. Bull. 198—after Chittenden.)

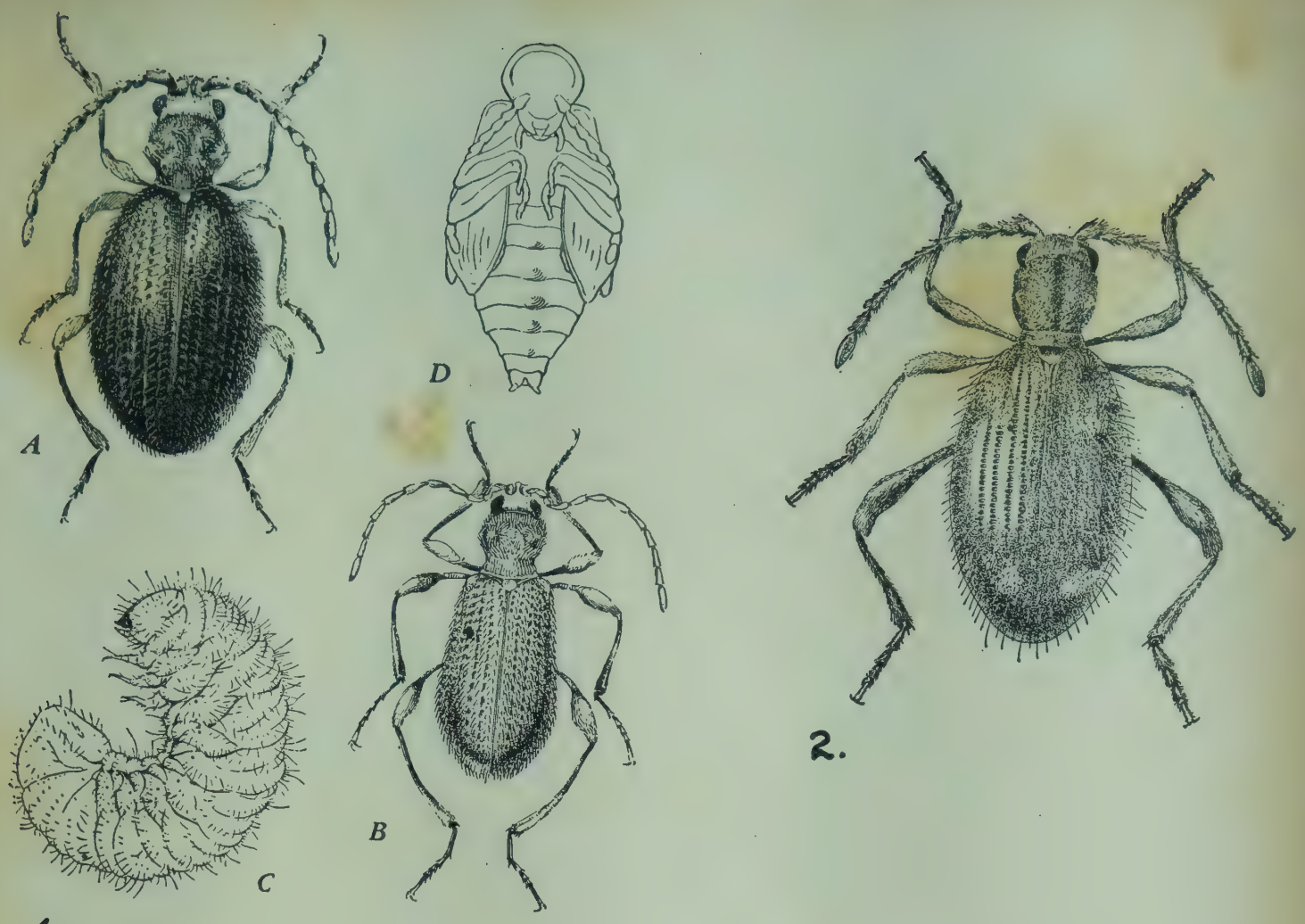


FIGURE 70.—1. Brown spider beetle; *A*, Adult female; *B*, Adult male; *C*, Larva; *D*, Pupa. 2. White marked spider beetle. (From: 1—California Agric. Expt. Sta. Bull. 676; 2—Farmers' Bull. 1260, U. S. Dept. Agric.)

made by cementing particles of food material. SPIDER BEETLES, *Ptinidae*.

[I, B, 1, a, (C)] (1) HAIRY SPIDER BEETLE, *Ptinus villiger*. (Figure 69, 4)

(2) WHITE MARKED SPIDER BEETLE, *Ptinus fur*. (Figure 70, 2)

(3) *Mezium americanum*. (Figure 72, 1)

(4) BROWN SPIDER BEETLE. (Figure 70, 1)

b. Adults less than half as broad as long; slender.

(A) Adult males with mandibles projecting out in front like horns.

(1) "Horns" stout; head fits into recess in the anterior edge of the pronotum. BROAD-HORNED FLOUR BEETLE, *Gnathocerus cornutus*. (Figure 70, 3) (Slide I–R–25)

(2) "Horns" slender; anterior edge pronotum practically straight. SLENDER-HORNED FLOUR BEETLE, *G. maxillosus*. (Figure 71, 1)

(B) Adults with no "horns."

(1) With head deflexed and hidden from above by the humped prothorax. BORERS, *Bostrichidae*.

(a) About 1/8" long; surface polished darker brown but somewhat roughened. LESSER GRAIN BORER, *Rhizopertha dominica*. (Figure 72, 2) (Slide I–R–2)

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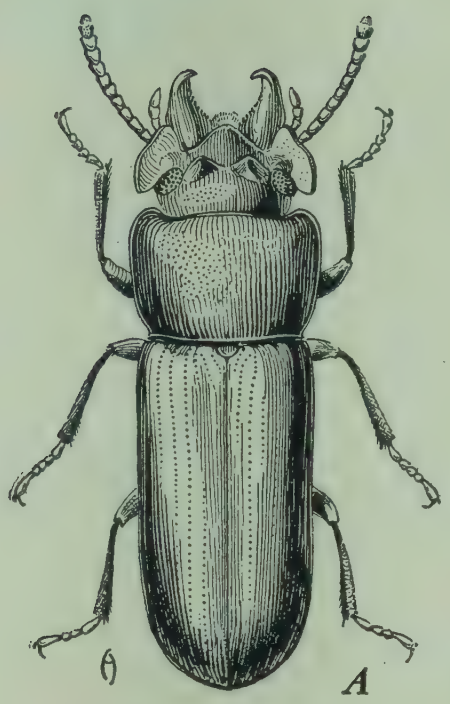

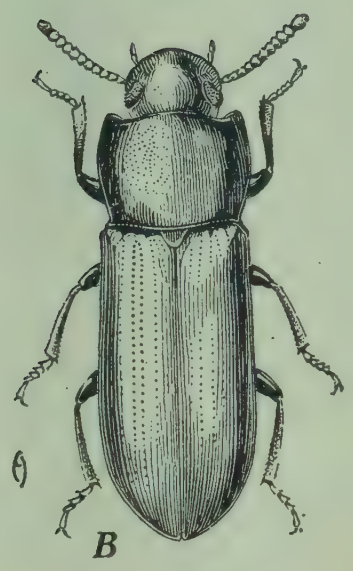

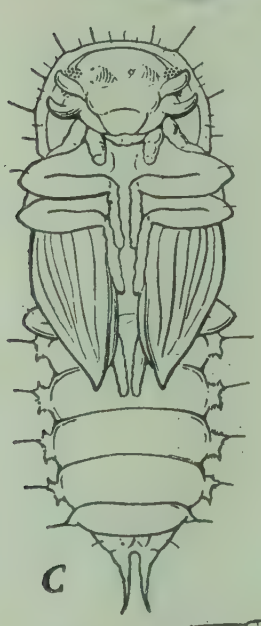

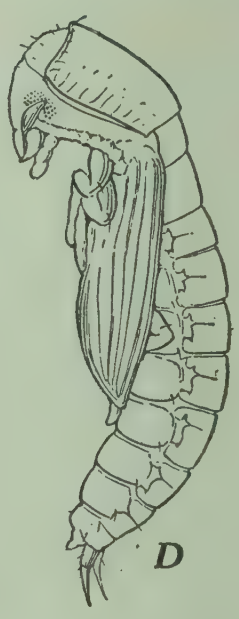

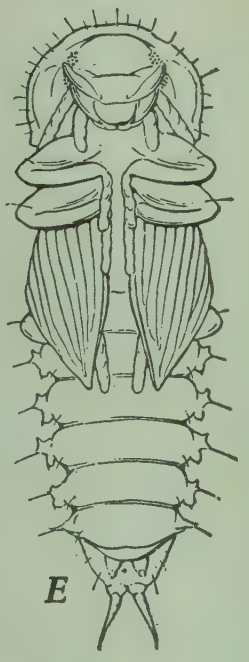

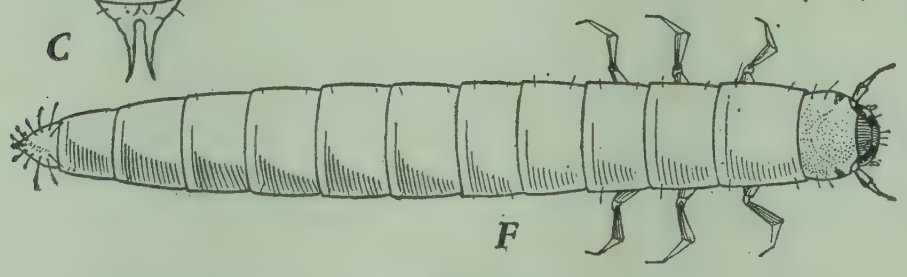

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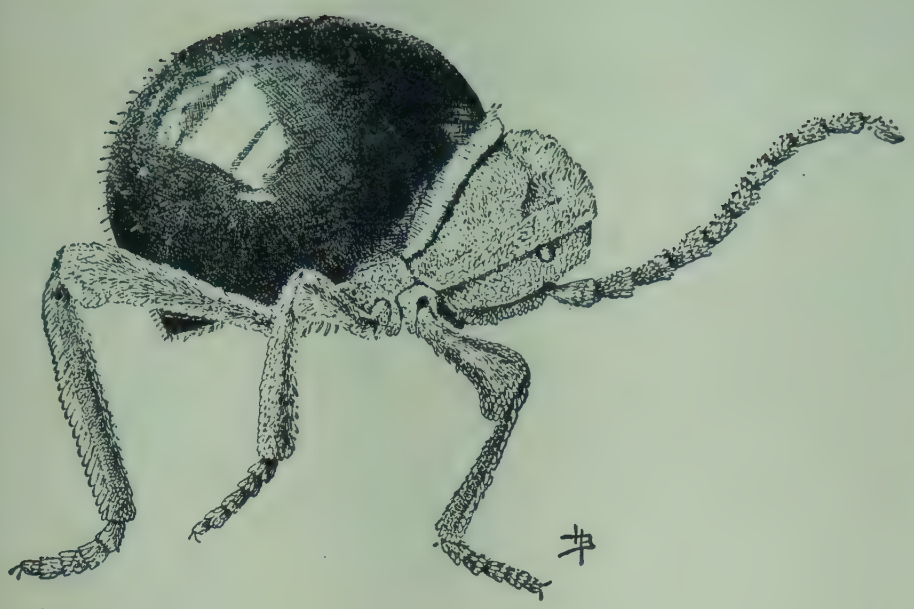
FIGURE 71.—1. Slender horned flour beetle. 2. Cigarette beetle. 3. Broad-horned flour beetle: *A,* Adult male; *B,* Adult female; *C,* Male pupa ventral aspect; *D,* Male pupa lateral; *E,* Female pupa ventral aspect; *F,* Larva. (From: 1, 2—Farmers' Bull. 1260, U. S. Dept. Agric.; 3—California Agric. Expt. Sta. Bull. 676.)



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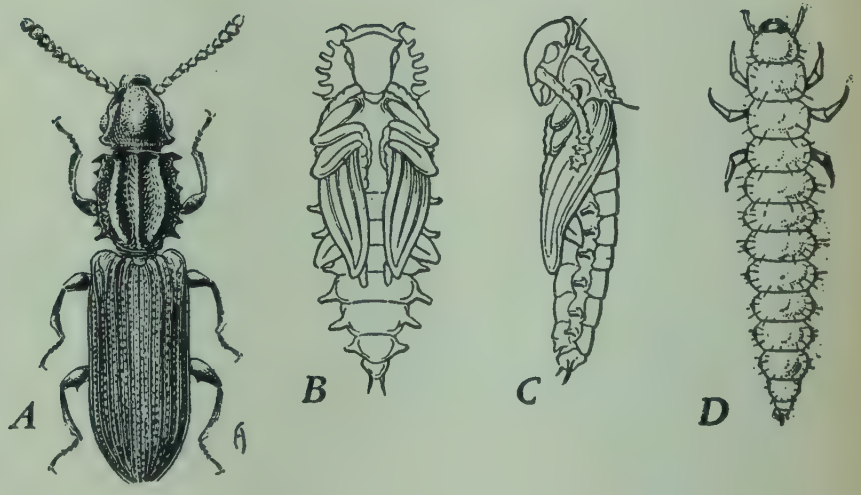

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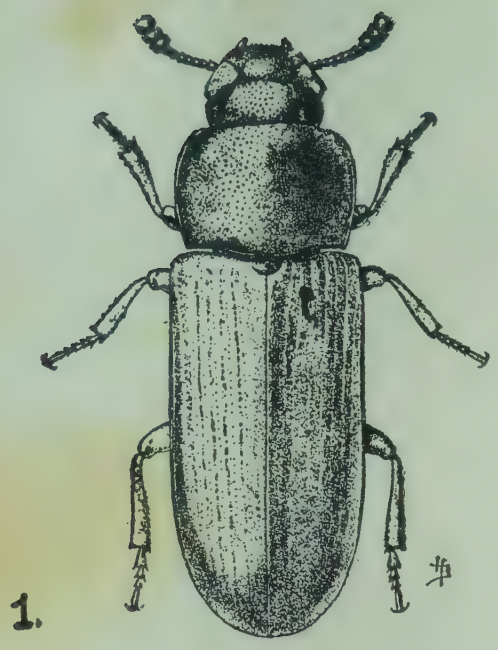
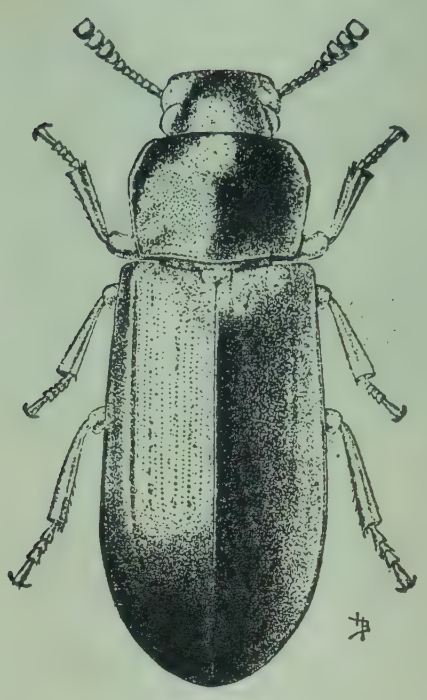
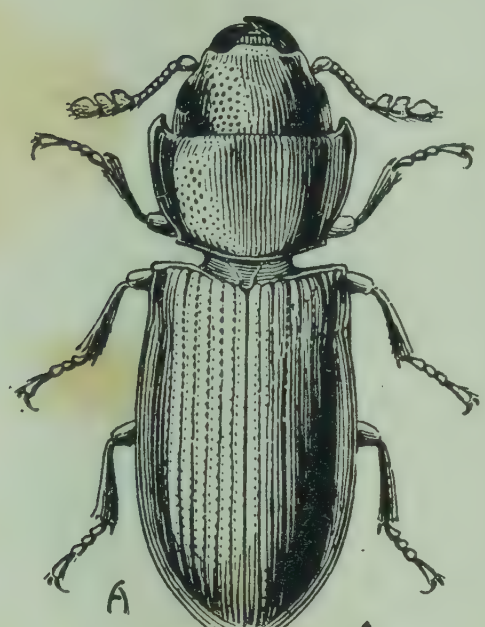
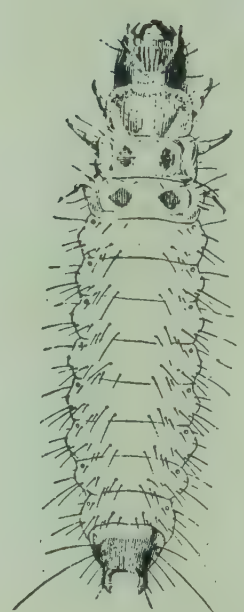
FIGURE 72.—1. *Mezium americanum*. 2. Lesser grain borer. 3. Larger grain borer. 4. Mexican grain beetle. 5. Foreign grain beetle. 6. Square necked grain beetle. 7. Sawtoothed grain beetle; *A*, Adult; *B* and *C*, Pupae; *D*, Larva. (From: 1, 3, 4, 5—Farmers' Bull. 1260, U. S. Dept. Agric.; 2, 6, 7—California Agric. Expt. Sta. Bull. 676.)









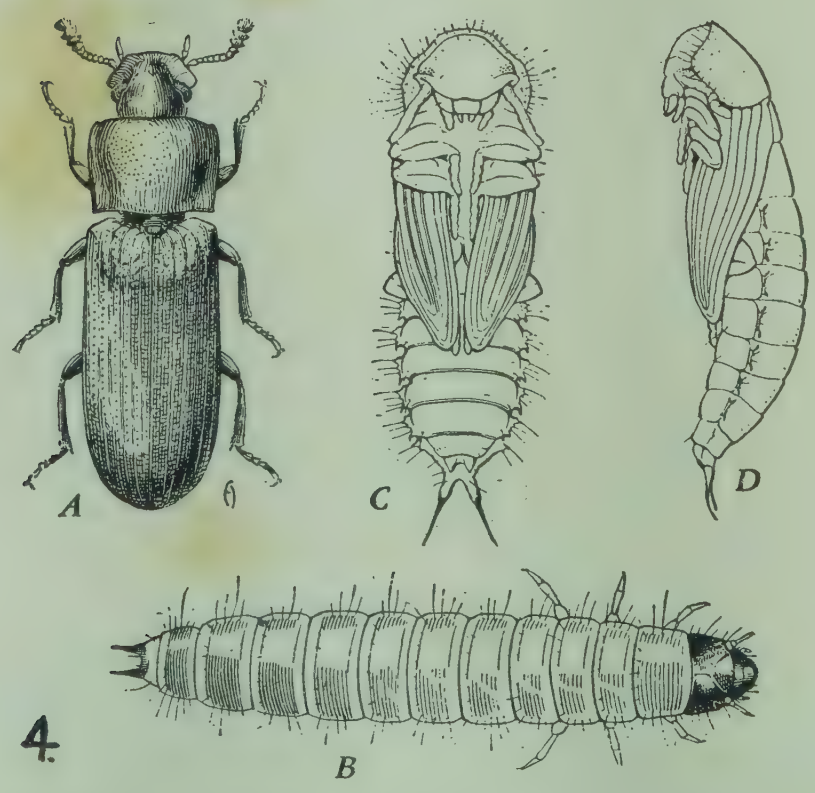
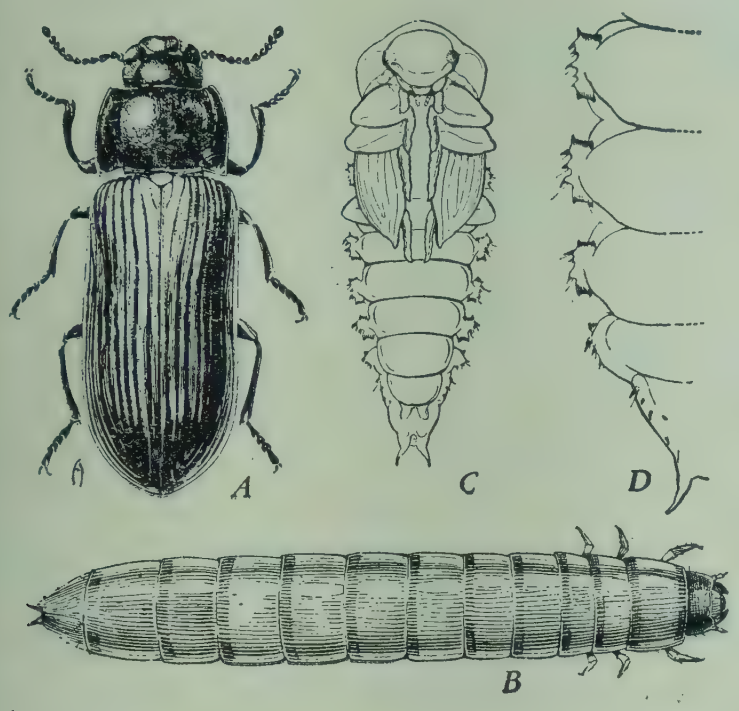


FIGURE 73.—1. Rust red flour beetle. 2. Black flour beetle. 3. Cadelle: *A*, Adult; *B*, Larva. 4. Confused flour beetle; *A*, Adult; *B*, Larva; *C*, Ventral view pupa; *D*, Lateral view pupa. (From: 1, 2—Farmers' Bull. 1260, U. S. Dept. Agric.; 3, 4—California Agric. Expt. Sta. Bull. 676.)

(b) About 1/6″ long; surface polished darker brown and comparatively smooth. LARGER GRAIN BORER, *Stephanopachys truncatus*. (Figure 72, 3)

[I, B, 1, b, (B),] (2) Head not relaxed; long and slender.

(a) "Teeth" on sides of thorax. SAWTOOTHED GRAIN BEETLE, *Orizaephilus surinamensis*. (Figure 72, 7) (Slide I–R–5)



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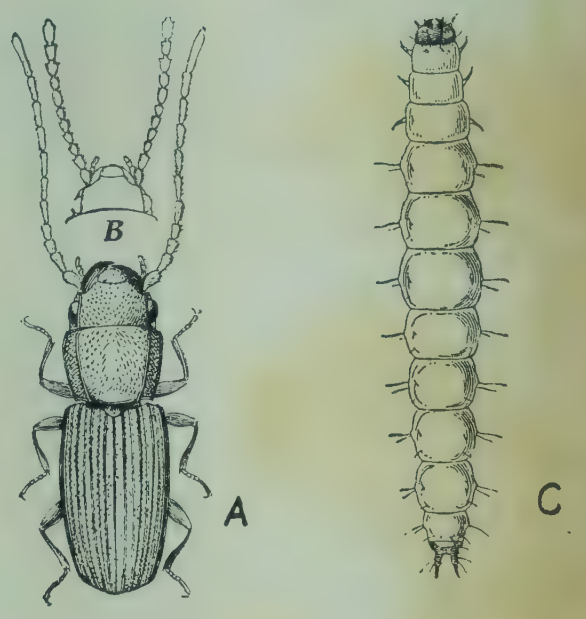

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FIGURE 74.—1. Yellow meal worm: *A*, Adult; *B*, Larva; *C*, Pupa; *D*, Lateral margin pupa abdomen. 2. Flat grain beetle: *A*, Adult male; *B*, adult female; *C*, Larvae. 3. Small eyed flour beetle. 4. Pea weevil. 5. Broadbean weevil. 6. Southern cowpea weevil. 7. Mexican bean weevil. 8. Cowpea weevil. 9. Bean weevil. (From: 1, 2—California Agric. Expt. Sta. Bull. 676; 3—Farmers' Bull. 1260; 4 to 9—Farmers' Bull. 1275, U. S. Dept. Agric.)

[I, B, 1, b, (B), (2)] (b) Thorax not "toothed."

A′. Adults; body not over 1/12″ long.

1′. Antennae slender; prothorax straight or outwardly rounded anteriorly.

a′. Antennae almost as long as body. FLAT GRAIN BEETLE, *Laemophlaeus minutus*. (Figure 74, 2). (Slide I–R–3)

b′. Male antennae as long or longer than body. *L. turcicus*.

c′. Male antennae not more than half as long as body. RUST-RED GRAIN BEETLE, *L. ferrugineus*.

2′. Antennae short and somewhat club-shaped; body somewhat rectangular; thorax recessed anteriorly where head fits in. SMALL EYED FLOUR BEETLE, *Palorus ratzeburgi*. (Figure 74, 3) Slide I–R–1)

B′. Adults; body over 3/32″ long.

1′. Adults long and slender, approximately 1/10″ long; thorax "square" the corners abruptly angular; thorax and abdomen have straight sides and are about the same width. SQUARE-NECKED GRAIN BEETLE. *Silvanus gemellatus*, (*Cathartus quadricollis*). (Figure 72, 6)

2′. Adults not so shaped; flattened and oval beetles about 3/16″ long with head and dorsal parts of thorax densely covered with minute punctures, and with elytra ridged lengthwise and sparsely punctured between the ridges; antennae not as long as head is wide. *Tribolium* sp.

a′. Prothoracic shield somewhat wedge-shaped, widening anteriorly, and giving a broad-shouldered effect; eye notched into head as seen from above; antennae segments gradually increase in size from base to tip. CONFUSED FLOUR BEETLE, *T. confusum*. (Figure 73, 4) (Slide I–R–15)

b′. Lateral edges of prothoracic shield rounded; margins of eye and head almost continuous; last few antennae segments abruptly larger than those at the base. RUST-RED FLOUR BEETLE, *T. castaneum*. (Figure 73, 1) (Slide I–R–7)

3′. Flattened, oval-elongated, somewhat deepened brown color; highly polished surface; antennae longer than either head or thorax is wide. MEXICAN GRAIN BEETLE, *Pharaxonotha kirschi*. (Figure 72, 4)

4′. More robust beetles; antennae enlarged at end; thorax with knob on anterior angles. FOREIGN GRAIN BEETLE, *Cathartus advena*. (Figure 72, 5)

[I, B] 2. Adults black, gray, bluish, or varied but not reddish brown.

a. Beetles about 1/6″ long; black; rather flattened dorso-ventrally, elongate-oval in shape. BLACK FLOUR BEETLE, *Tribolium madens*. (Figure 73, 2)

b. Large black beetles over 1/4″ long. Larvae 1/2″ long or longer when full grown.

(A) Adults 1/2″ long; two clefts on posterior edge of prothoracic shield. Larvae yellow to dark brown with a hard exoskeleton. MEALWORM, *Tenebrio* sp. (Figure 74, 1)

(B) Adults slightly more than 1/4″ long. Larvae have a tough pigmented forked plate at the posterior end. CADELLE, *Tenebriodes mauritanicus*. (Figure 73, 3) (Slide I–R–6)

c. Smaller beetles less than 1/4″ long, bluish black, gray, or varied, well rounded, not flattened.

(A) Plump gray beetles found in peas and beans; approximately 1/8″ long, wing covers mottled with light and dark spots. Larvae legless grubs. Somewhat weevil-like in their habits and appearance, but no really elongated snout present. *Bruchus* (*Mylabrus*) sp.

(1) Small but distinct white spots on the elytra; thorax broad. PEA WEEVIL, *B. pisorum*. (Figure 74, 4)

(2) Similar to the Pea Weevil except that the anterior pronotum edge is almost perfectly rounded; thorax narrower. BROADBEAN WEEVIL, *B. rufimonus*. (Figure 74, 5)

(3) Mottling indistinct; thorax somewhat cone-shaped. BEAN WEEVIL, *B. obtectus*. (Figure 74, 9)

(4) Mottling in a pattern of four black spots on the elytra; thorax cone-shaped. SOUTHERN COWPEA WEEVIL, *B. quadrimaculatus*. (Figure 74, 6)

(5) A light band extending across each elytra. MEXICAN BEAN WEEVIL, *Spermophagus pectoralis*. (Figure 74, 7)

(6) Two ivory-like spots near the mid-dorsal line where the elytra join the thorax; antennae deeply toothed; posterior end of body squared off abruptly. COWPEA WEEVIL, *B. chinensis*. (Figure 74, 8)

(B) Black, dark brown, bluish, or vari-colored.

(1) Adults small; antennae with stout basal and terminal segments and very narrow segments along most of the length. Larvae with few hairs.

(a) Slightly over 1/8″ long; black with a pronounced banding and two pale brown spots on each elytron; pale antennae and legs; short truncated elytron. Larvae with two pairs of tubercles at the posterior end. DRIED FRUIT BEETLE, *Carpophilus hemipterous*. (Figure 75, 1) (Slide I–R–37)

(b) Slightly less than 1/8″ long; dark brown with lighter elytra; short truncated elytra. CORN SAP BEETLE. *C. dimidiatus*. (Figure 76, 5)

[I, B, 2, c, (B)] (2) Larvae clothed with short scale-like hairs and with a long tuft of hairs at the posterior end of the body; often with several erect dorsal tufts. Adult antennae not as in "(1)" although that arrangement may be present to a much less noticeable degree; often covered with a hairy pubescence. DERMESTIDS.

(a) Adults dark brown or black with a light band across the middle of the body. LARDER BEETLE, *Dermestes lardarius*. (Figure 76, 4)

(b) Adults small, black mottled with reddish brown and covered with gray and light brown hairs. LARGER CABINET BEETLE, *Trogoderma versicolor*. (Figure 76, 1)

(c) Adults black. Larvae reddish or golden brown. BLACK CARPET BEETLE, *Attagenus piceus*. (Figure 76, 2)

(d) Adults black with yellowish-white scales forming a broad band across the back. VARIED CARPET BEETLE, *Anthrenus verbasci*. (Figure 75, 2)

(e) Adults black, marked with reddish, white, and yellow spots. Larvae very hairy but with the terminal brush inconspicuous. BUFFALO CARPET BEETLE, *A. scrophulariae*. (Figure 76, 3)

(3) Adults, iridescent steel blue; 1/5" long; legs reddish; head and prothorax finely pubescent. RED LEGGED HAM BEETLE, *Necrobia rufipes*. (Figure 77, 1)

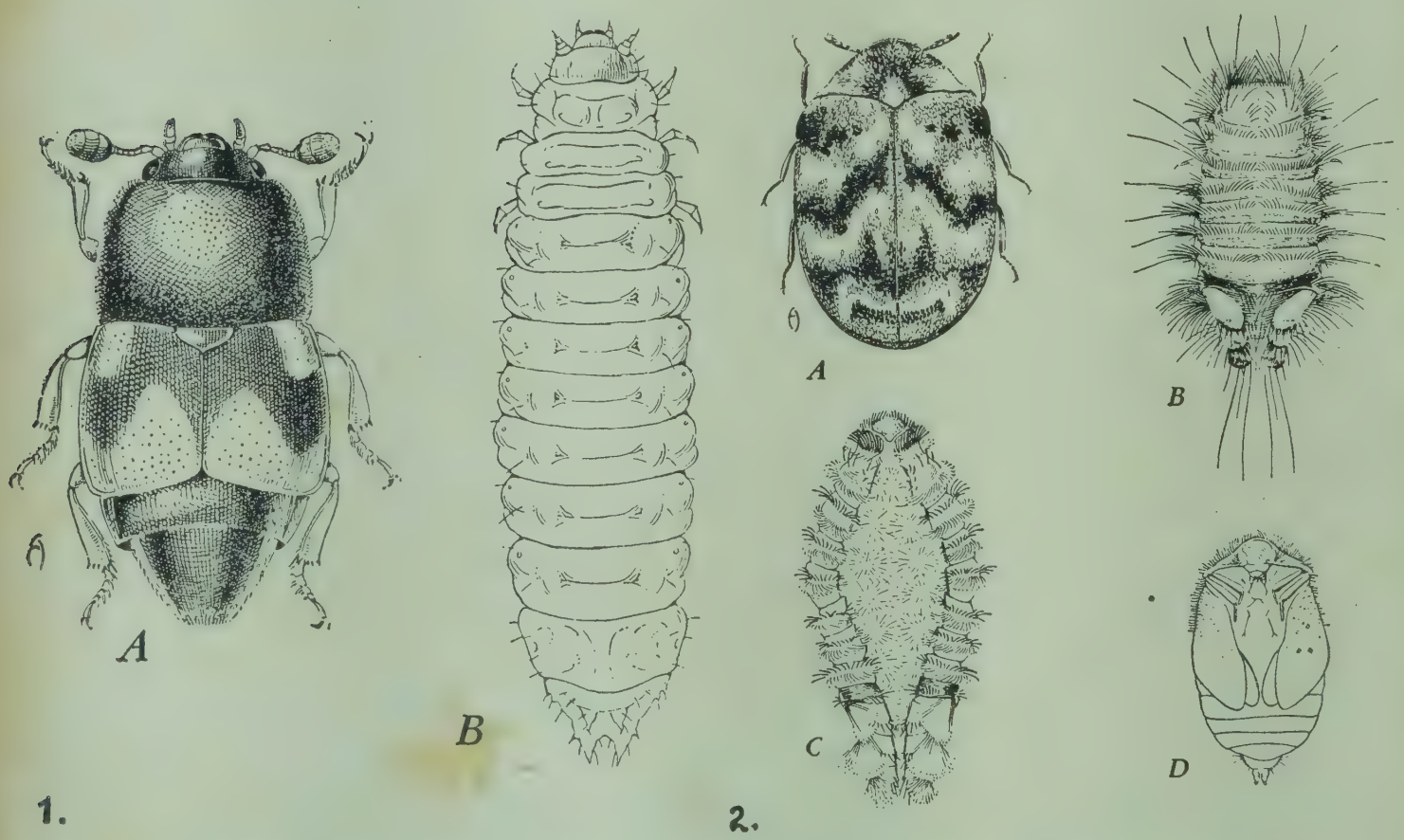
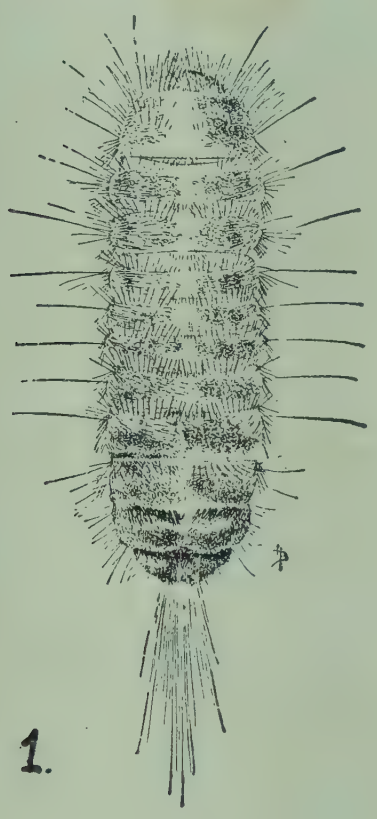


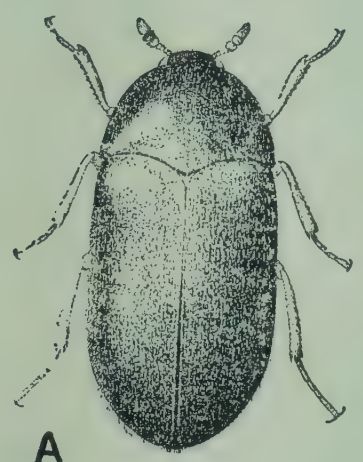
FIGURE 75.—1. Dried fruit beetle: *A*, Adult; *B*, Larva. 2. Varied carpet beetle: *A*, Adult; *B*, Larva; *C*, Pupa within larval skin; *D*, Pupa removed from skin. (From California Agric. Expt. Sta. Bull. 676.)

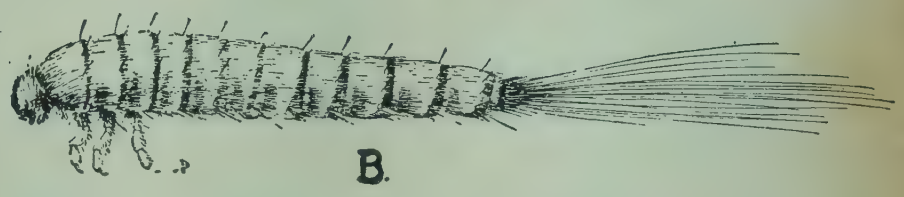
[I, B] 3. Adults pale yellow to yellowish brown, barrel-shaped antennae segments; antennae club-shaped with the last segment smaller; minute canthus behind each eye; head appears elongated. LONG HEADED FLOUR BEETLE, *Latheticus oryzae.* (Figure 77, 2)

II. Adults with four wings covered with dust-like scales; body and legs also covered with scales. Larvae with more than three pairs of legs, i.e. with four or more pairs of sucker-like pseudopods in addition to the thoracic true feet. MOTHS AND CATERPILLARS, LEPIDOPTERA. (The colorings and patterns on moth wings are formed by the scales. These scales are readily rubbed off and the typical patterns are most obvious in a newly emerged adult, and may become very indistinct within a few days.)

A. Narrow pointed forewings, with pronounced fringe of hairs; wing expanse about ½″.







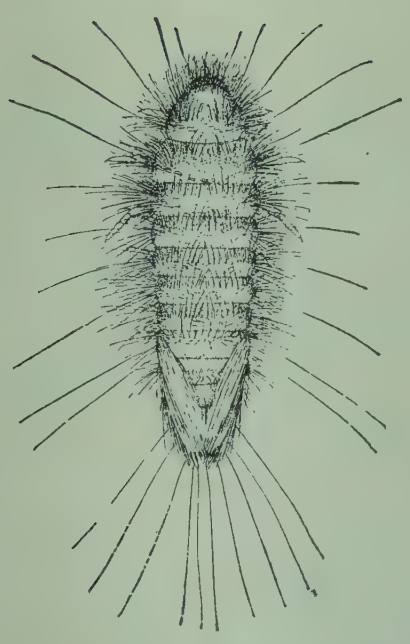
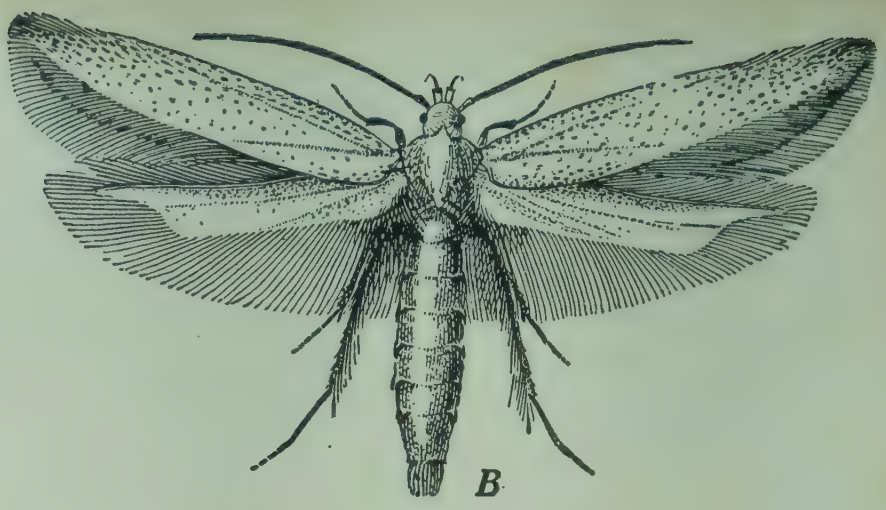


FIGURE 73.—1. Larger cabinet beetle larva. 2. Black carpet beetle: *A,* adult; *B,* Larva. 3. Buffalo carpet beetle: *A,* Larva; *B,* Adult. 4. Larder beetle. 5. Corn sap beetle. (From: 1, 2*B,* 5—Farmers' Bull. 1260, U. S. Dept. Agric.; 2*A,* Connecticut Agric. Expt. Sta. Bull. 400; 3—California Agric. Expt. Sta. Bull. 676; 4—Minnesota Agric. Expt. Sta. Bull. 341.)

1.



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A C D

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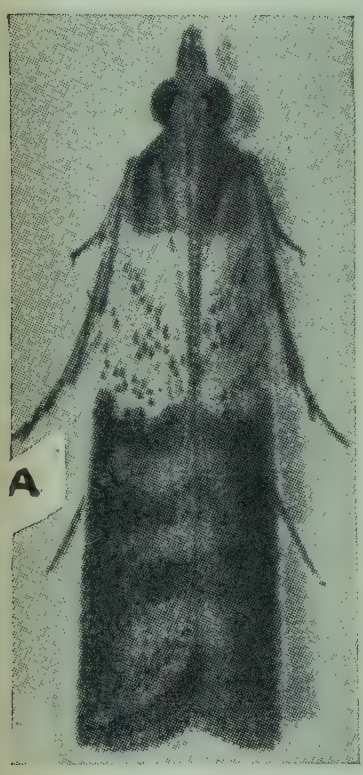
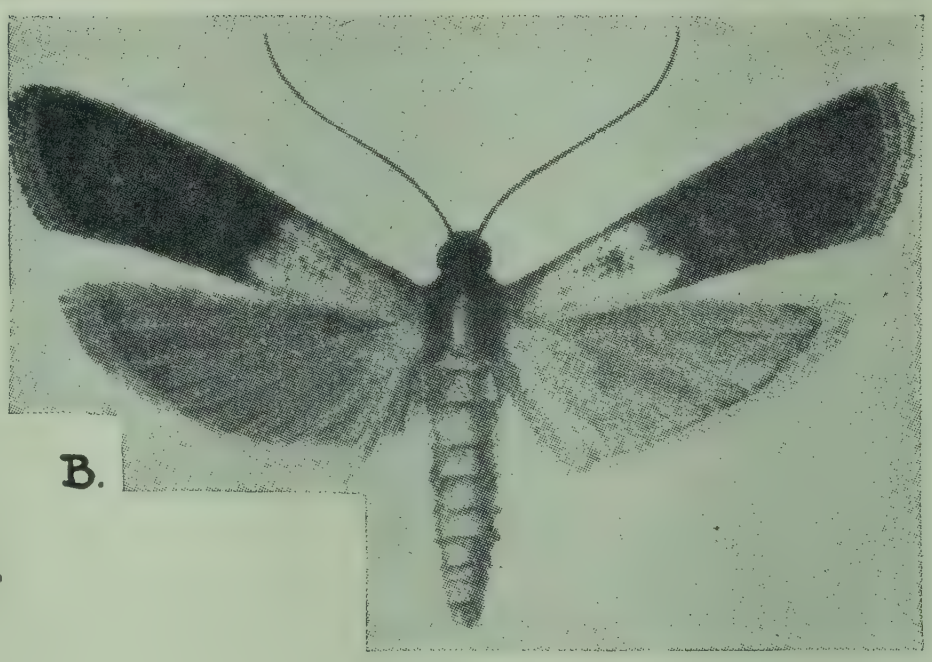


A



B.

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FIGURE 77.—1. Red legged ham beetle. 2. Long headed beetle. 3. Angoumois grain moth: *A,* Adult in normal position; *B,* Adult with wings spread; *C,* Larva; *D,* Pupa. 4. Indian meal moth: *A,* Adult in resting position; *B,* Adult with wings spread. (From: 1—Minnesota Agric. Expt. Sta. Bull. 198; 2, 4—Farmers' Bull. 1260, U. S. Dept. Agric.; 3—California Agric. Expt. Sta. Bull. 676.)

[II, A] 1. Fore wings mottled.

a. Hind wings of "normal" width; gray and brown mottling on fore wings. EUROPEAN GRAIN MOTH, *Nemapogon granella.* (Figure 78, 3)

b. Hind wings very slender, approximately ¼ the width of the fore; fore wings mottled with yellow, reddish-brown, and black. PINK CORN WORM, *Pyroderces rileyi.* (Figure 79, 2)

[II, A] 2. Fore wings evenly colored. Larvae are the only caterpillars found within grain. ANGOUMOIS GRAIN MOTH, *Sitotroga cerealella*. (Figure 77, 3)

B. Fore wings not pointed.

1. Wing expanse not over 9/16″, pale grayish brown or yellowish brown; no variegated wing markings. RICE MOTH, *Corcyra cephalonica*. (Figure 79, 1)

2. Wing expanse approximately 3/4″–1″.

a. Inner half of fore wings light brown, outer half dark with a coppery lustre. Larvae yellowish, green, or pink; leave webbing

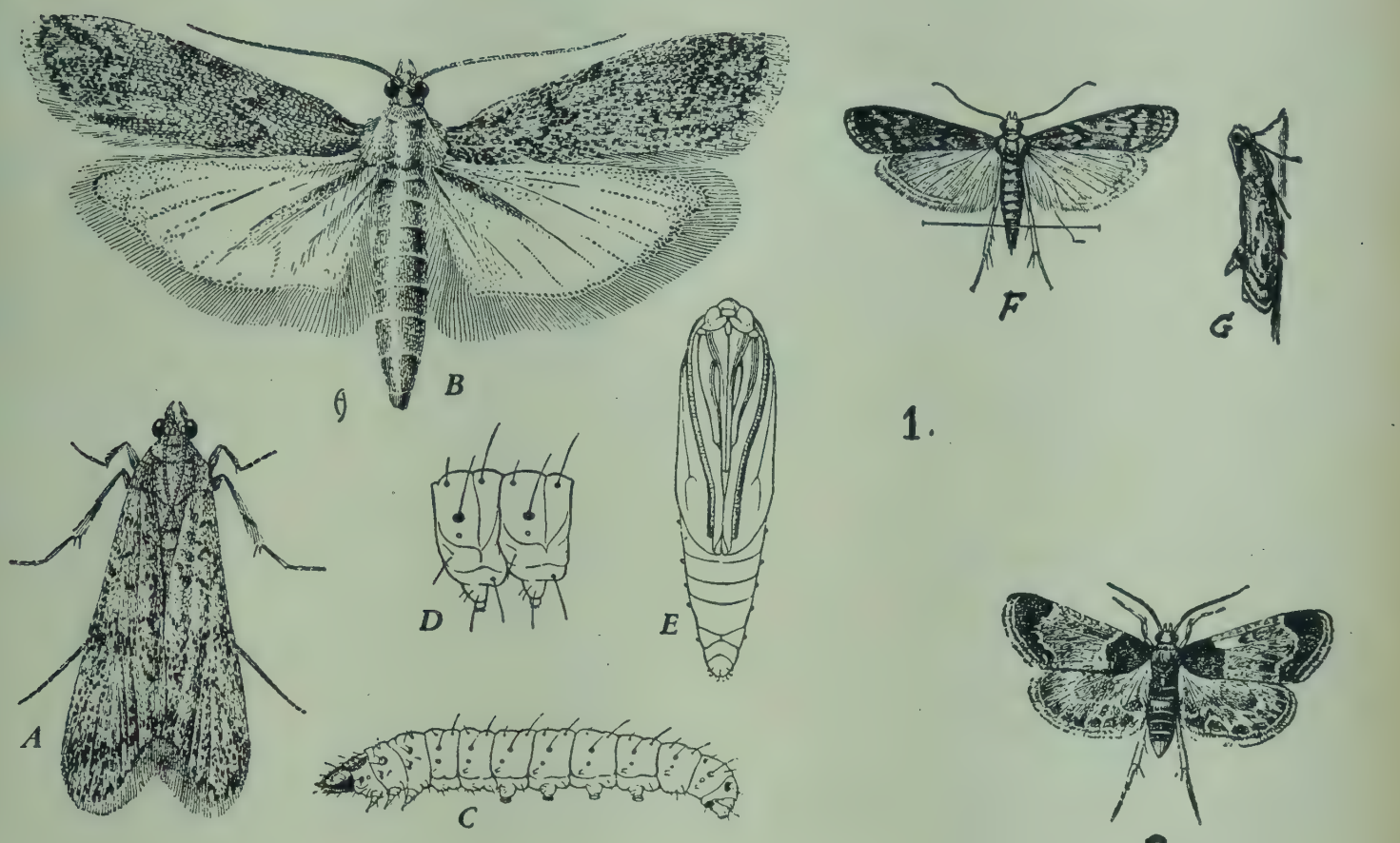


FIGURE 78.—1. Mediterranean flour moth: *A*, Adult in normal resting position; *B*, Adult with wings spread; *C*, Larva; *D*, Two segments of the larval thorax; *E*, Pupa; *F*, Adult freshly emerged and scales intact showing more vivid wing markings; *G*, Adult side view showing arched position. 2. Meal snout moth. 3. European grain moth. 4. Fig moth. (From: 1. *A*, *B*, *C*, *D*, *E*—California Agric. Expt. Sta. Bull. 676; 1. *F*, *G*, 3—Farmers' Bull. 1260, U. S. Dept. Agric.; 2—Minnesota Agric. Expt. Sta. Bull. 341—after Chittenden; 4—Bur. Ent. Bull. 104, U. S. Dept. Agric.)

in and over the food, but do not live in a silk tube. INDIAN MEAL MOTH, *Plodia interpunctella*. (Figure 77, 4) (Slide I–R–9)

[II, B, 2] b. Fore wings dark chocolate brown at both ends, middle dusky white with two wavy white lines separating the lighter and darker areas. Larvae solid grayish, darker at the ends; web the food together and live in a silk tube. MEAL SNOUT MOTH, *Pyralis farinalis*. (Figure 78, 2)

c. Wing color grey-brown with transverse dark bars; bars become indistinct when the wings become worn; at rest the moth elevates the fore part of the body so that the wings have a distinct slope and are wrapped around the body. Yellowish, white, or pinkish larvae spin silk and often web the food together in irregular masses. *Ephestia* sp. (By gross morphological characters alone, it is difficult to distinguish between the species of this genus.)

(A) Fore wings pale in the basal half and more gray to chocolate brown in the apical half; wing expanse approximately $\frac{3}{16}$–$\frac{5}{16}$″. CHOCOLATE MOTH, *E. elutella*.

(B) Fore wings with fairly pronounced markings; markings transverse, black, zigzag; wing expanse approximately $\frac{7}{8}$–1″. MEDITERRANEAN FLOUR MOTH, *E. kuehniella*. (Figure 78, 1) (Slide I–R–29)

(C) Markings of the fore wings much more suffused; the line across the basal third is whitish, more nearly straight and bordered by a prominent dark, suffused band. In the others this line is irregularly dentated or zigzag; wing expanse approximately $\frac{9}{16}$–$\frac{13}{16}$″. FIG MOTH, *E. cautella*. (Figure 78, 4)

III. Fore wings leathery; long legs; long, slender, tapering antennae; bodies thin, flat and regularly oval in shape; active runners. COCKROACHES, Orthoptera.

A. Length not over $\frac{5}{8}$″; color light brown with two dark brown stripes on the prothorax. GERMAN ROACH, CROTON BUG, WATER BUG, *Blatella germanica*. (Figure 79, 3) (Slide I–R–22)

B. More than 1″ long; color black, chocolate brown to red brown.

1. Wings truncated; in the male extending $\frac{2}{3}$ the abdomen length; in the female extending less than $\frac{1}{4}$ the abdomen length, and with no hind wing; dark brown to black. ORIENTAL COCKROACH. *Blatta orientalis*. (Figure 79, 4)

2. Wings fully developed and covering the entire abdomen; length 1½–2″.

a. Uniform brown color except for markings on the prothoracic shield. AMERICAN COCKROACH, *Periplaneta americana*. (Figure 80, 1)

b. Yellow line on the outer edge of the basal half of the fore wing. AUSTRALIAN COCKROACH, *P. australaciae*. (Figure 80, 2)

IV. No wings.

A. Naked.

1. Marked constrictions between body regions; hard external

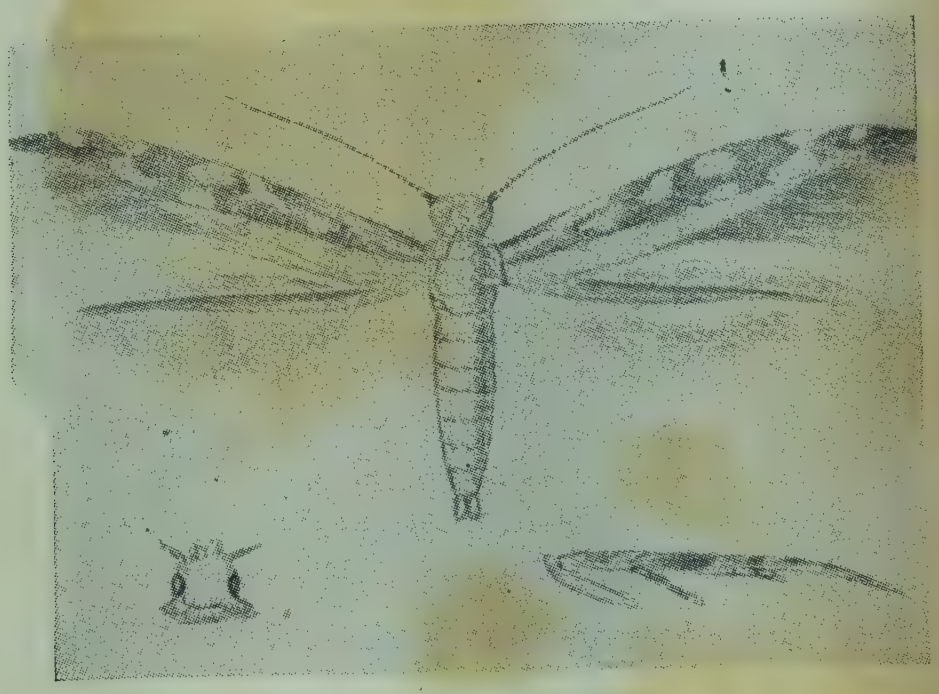
covering; petiole between the thorax and the abdomen variously shaped and humped; long legs and slender bodies; antennae elbowed. ANTS. (Figure 66, *A*) (Slide I–R–32 & 33)

[IV, A] 2. Minute, scarcely larger than a pinhead; flattened; broad heads; robust broadly-joined body. *Psocids*. (Figure 59, *B*)

B. Covered with dust-like scales; somewhat larviform in shape; long antennae, and long cerci at the posterior end. THYSANURA.

1. Silvery-gray or brownish. SILVER FISH, *Lepisma saccharina*. (Figure 80, 3)

2. Decidedly mottled gray in color. FIREBRAT, *Thermobia domestica*. (Figure 80, 4)



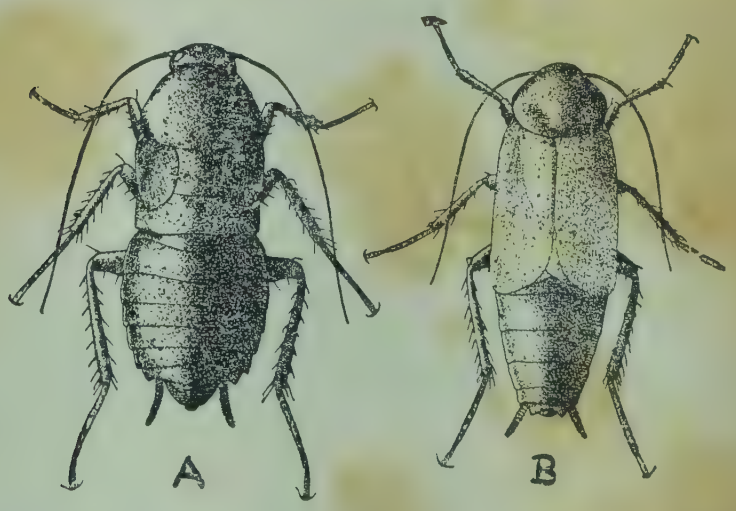


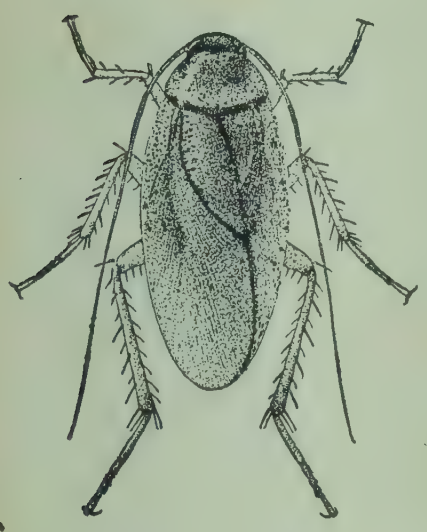
FIGURE 79.—1. Rice moth. 2. Pink corn worm. 3. German roach. 4. Oriental roach: *A*, Female; *B*, Male. (From: 1, 2—Farmers' Bull. 1260, U. S. Dept. Agric.; 3, 4—Connecticut Agric. Expt. Sta. Bull. 400.)

V. Both pairs of wings membraneous.

A. One pair of wings, the hind pair represented by a pair of knob-like halters. The larvae are legless maggots. FLIES. (Note: most flies (Slide I–R–17 & 23) found in foods do not represent true "storage infestations." An exception to this is the case of the CHEESE SKIPPER, *Pipohila casei*, a small dark bronze or bluish-black fly with the face, mouth-parts, and antennae yellow. The maggots jump freely.)

B. Two pairs of wings. Various hymenoptera. None of the hymenoptera are storage pests, but many of them are parasitic upon food-infesting insects and so may be found in foods.

VI. Not true insects, minute, 8-legged arachnids with sparsely scattered but elongate hairs; head, thorax, and abdomen all joined in one body region. MITES. (Slide I–1)



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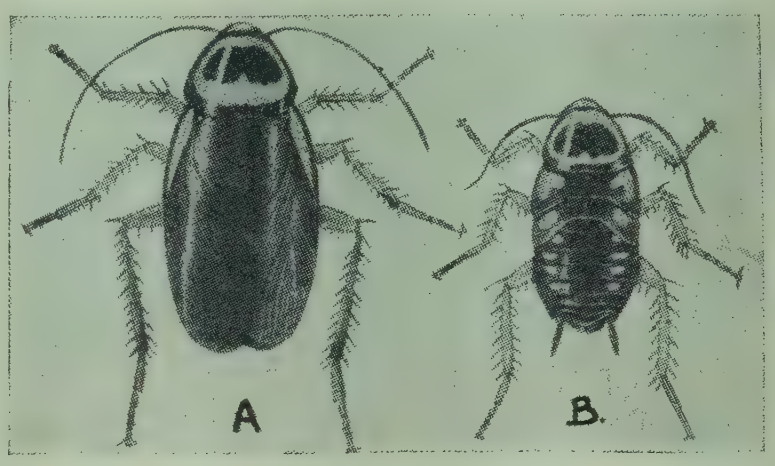

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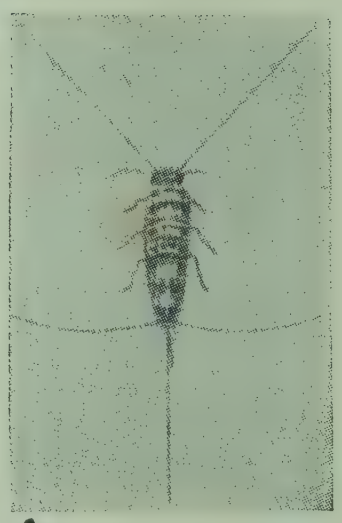

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FIGURE 80.—1. American cockroach. 2. Australian cockroach: *A*, Adult; *B*, Nymph. 3. Silverfish. 4. Firebrat. (From: 1—Connecticut Agric. Expt. Sta. Bull. 400; 2—Leaflet 144, U. S. Dept. Agric.; 3, 4—Farmers' Bull. 1260, U. S. Dept. Agric.)

E. Foods and Their Common Insect Contaminants

In order to include more species than was practical in following the somewhat abbreviated listing given in the foregoing descriptive material, this basic discussion was enlarged by the inclusion of references to a standard textbook of entomology. "College Entomology" by E. O. Essig, the Macmillan Company, New York, 1942, is used as the basic reference for several reasons, the most important of which is that its pedagogic style and outline and tabular form lend themselves to a good deal of self-teaching. This book is basically an introduction to taxonomy; it employs every-day language, common names of insects, and generalized descriptions of various insect groups. Such books as "Destructive and Useful Insects" by C. L. Metcalf and W. P. Flint, McGraw-Hill, New York, 1939, and "Manual of Injurious Insects" by Glenn W. Herrick, Henry Holt & Company, New York, 1925, contain arrangements of insects destructive to various food crops and should be used along with the references in this discussion and with "College Entomology."

Practically all insects have parasites which prey upon them. While a listing of parasites that may be associated with insects contaminating foods is beyond the scope of this work, most of them are members of a relatively small group of hymenoptera and the adults, when found, can be recognized as such. (See Essig, pages 619 and ff.)

1. List of References.

Numbers prefixed with an "E" are page references to Essig's "College Entomology," "I" and "I–R" numbers refer to authentic slide material, and "K" indicates that it is listed in the key to storage pests.

1. BEVERAGES, LEAFY CRUDE DRUGS AND CONDIMENTS:

Citrus juice: Fly eggs and maggots, E–734, E–793; scale insects, E–354; I–R–18.
Coffee: Coffee berry beetle, E–605; see Cereals for storage pests.
Copra: Cigarette beetle, E–577; see Cereals for storage pests.
Cocoa: See Cereals for storage pests.
Drugs, crude leafy: Aphids, E–331; thrips, E–258; see Cereals for storage pests.

2. CEREALS, GRAINS, BEAN SEEDS, MOST STORED DRIED FOODS, BAKERY PRODUCTS:

American cockroach, K.
Angoumois grain moth, E–458; K.
Ants, E–667; I–R–32; I–R–33.
Aphids, E–331.
Australian cockroach, K.
Bean weevils, K.
Black carpet beetle, K.
Black flour beetle, K.
Broad-horned flour beetle, E–573; I–R–25; K.
Broadnosed grain weevil, K.
Buffalo carpet beetle, K.
Cadelle, E–562; I–R–6; I–R–16; K.
Chocolate moth, E–468; K.
Cigarette beetle, E–577; I–R–19; K.
Cockroaches, E–109; K.

2. CEREALS, etc. (continued):
Pea weevil, K.
Pink corn worm, K.
Psocids, E–184; K.
Raisin moth, E–468.
Red legged ham beetle, K.
Rice moth, K.
Rice weevil, I–R–4; K.
Rust-red flour beetle, E–573; I–R–7; I–R–10; K.
Rust-red grain beetle, K.
Sawtoothed grain beetle, E–563; I–R–5; I–R–43; K.
Silverfish, E–69; K.
Slender horned flour beetle, K.
Small eyed flour beetle, I–R–1; I–R–11; K.
Spider beetle, E–577; K.
Weevils, E–595; E–602.

3. CONFECTIONERY, SUGARS, SIRUPS, HONEY:
(For storage pests of candy, copra, chocolate, etc., see Cereals.)
Ants, E–667.
Beemoth (honey), E–467.
Flies, E–728.
Honeybee (honey), E–714.
Lesser waxworm (honey), E–467.
Mites, (see California Circular #87)
Confused flour beetle, E–573; I–R–13; I–R–15; I–R–27; K.
Corn sap beetle, K.
Dermestids, E–559; K.
Dried fruit beetle, I–R–37; I–R–38; K.
Drugstore beetle, E–577; I–R–26; K.
Ephestia spp., K.
European grain moth, K.
Fig moth, E–468; K.
Fire brat, E–69; K.
Flat grain beetle, I–R–3; K.
Foreign grain beetle, K.
German 'roach, I–R–22; K.
Granary weevil, I–R–8; K.
Housefly, I–R–17; I–R–23; I–R–28
Indian meal moth, E–468; I–R–9; I–R–42; K
Larder beetle, K.
Larger cabinet beetle, K.
Larger grain borer, K.
Lesser grain borer, E–576; I–R–2; K.
Meal snout moth, K.
Mealworm, E–573; K.
Mediterranean flour moth, E–468; I–R–29; K.
Mexican grain beetle, K.
Mites, (see California Circular #87)
Meal moth, E–465.
Oriental roach, K.

4. DAIRY PRODUCTS:
(For storage pests of cheese and dried dairy products see Cereals.)
Cheese skipper, E–788; K.
Flies, E–728.

5. & 6. EGGS AND FISH:
(For storage pests of dried eggs and fish see Cereals.)

7. SPICES:
(For storage pests of spices see Cereals.)
(For pests on spice leaves see Truck crops.
Related species are to be found on all plant leaves.)

PICKLES AND RELISHES:
Rat-tailed maggot, E–782.
(See also pests of the raw product.)

8. FRUITS:
(For storage pests of dried fruits see Cereals.)
General feeders of fruits, fruit juices and fruit pomace:
Borers, E–455; fruit flies, E–789; rat-tailed maggots, E–782; scales, E–352; E–356; E–358; thrips, E–258; vinegar flies, E–793.
Apple: Codling moth, E–460; maggot, E–791; worm, E–461.
Berries: Blueberry maggot, E–791; I–R–24; *Byturus* sp., E–560; cherry fruitfly, E–791; cherry sawfly, E–631; currant fly, E–791.
Fig: Blastophaga, E–654.
Grape: Grape berry moth, E–461.
Olive: Olive fly, E–791.
Peach: Peach borer, E–455; peach moth, E–461; twig borer, E–458.
Strawberry: Strawberry crown borer, E–455.

11. NUTS:
Codling moth, E–460. (For storage pests of nuts see Cereals.)

13. VEGETABLES:
(For storage pests of dried vegetables see Cereals.)
General feeders on soft rotten produce: Carrion beetle, E–545; housefly, E–799; rove beetles, E–546; vinegar flies, E–793.
Mushrooms: (For figure of mushroom pests see Figure 82); I–1; collembola, E–77; mushroom fly, E–748. (For more complete details see discussion at the end of this section.)
Truck crops: Aphids, E–331; bugs, E–263; E–275; E–331; caterpillars, E–475, E–483, E–498; centipedes, (see California Circular #87); collembola, E–84; grasshoppers, katydids and crickets, E–87, E–95, E–98; lace bugs, E–287; leafhoppers, E–318; leafminers, E–794; mites, (see California Circular #87); mole crickets, E–102; stink bugs, E–275; thrips, E–258; white flies, E–322; wire worms, E-555.
Asparagus: Beetle, (see California Circular #87); fly, E–791.
Beans: Mexican bean beetle, E–569; pod borer, E–468.
Beets: Leafminer, E–797; wireworms, E–555.
Broccoli: (See Brussels sprouts).
Brussels sprouts: Cabbage aphid, E–339; I–R–14; cabbage bug, E–276; cabbage looper, E–474; cabbage maggot, E–797; cabbage worms, E–506.

13. VEGETABLES (continued):
Cabbage: (See Brussels sprouts).
Carrot: Rust fly, E–788; wireworms, E–555.
Corn: Corn earworm, E–476.
Cowpeas: Cowpea curculio, I–R–12.
Curcurbitae: Borers, E–455; melon fly, E–791.
Onion: Maggot, E–796; thrips E–256.
Pea: Aphid, E–339; moth, E–461; pod borer, E–468.
Potato: Tuber moth, E–457.
Pumpkin: Bug, E–277.
Spinach: (See aphids and thrips, above); leafminer, E–797.
Squash: (See cururbitae, above).
Tomato: Corn earworm, E–476; pinworm, E–458; Sphinx worm, E–481; E–483; potato tuber moth, E–457–8.

MUSHROOM INSECTS

Mushrooms, both the common cultivated type and the wild species, are subject to insect infestation. The wild species are, however, more subject to infestation since they are grown in the woods and fields and there are no adequate means of control as in the case of the cultivated types. Wild species of mushrooms are used for drying and constitute the commercial product, "dried mushrooms." Since these mushrooms are subject to a large amount of insect infestation, many forms of insects are frequently found in the examination of this type of product. Formerly large amounts of dried mushrooms were imported into this country from China, Japan, Yugoslavia, and some European countries. According to Maule and Hannum (1935) some 455,000 pounds of dried mushrooms were imported in 1934 from Europe. At the present time, some domestic wild species are being used for the dried product. The commercially grown, cultivated mushroom is marketed mainly as fresh or canned mushrooms. These products are relatively free of insect contamination since considerable care is given to their preparation.

The insects commonly encountered include:

COMMON NAME	SCIENTIFIC NAME
Mushroom Flies (Fungus gnat)	*Sciara coprophila*
	Sciara agraria
	Sciara multiseta
Manwie Flies (Phorids)	*Megaselia albidohalteris*
	Megaselia agarici
Gall Gnats (Cecid fly)	*Mycophila fungicola*
Silver Springtail	*Lepidocyrtus cyaneus*
Gray Springtail	*Achorutes armatus*
Mushroom Mites	*Tyroglyphus longior*
	Tyroglyphus lintneri
Flat-footed Flies (Diptera)	*Platypeza* sp.

FUNGUS GNATS.—The greatest amount of damage to mushrooms is done by maggots of flies (Figure 81). These maggots, commonly called larvae, feed on the mushrooms producing tunneling in the stems and caps. The most important of these flies is the FUNGUS GNAT. There are

at least three species of fungus gnats belonging to the Diptera insects in the family Sciaridae and genus *Sciara*. The adult flies are slender, black or brown, with long, thin yellowish or blackish legs, long wings and long slender antennae. The adults are not commonly found in the dried product. The larvae or maggots are white and transparent and the dark contents of their food canals can be seen through their body walls. These maggots have dark, chitinous heads which serve to distinguish them from other kinds of fly maggots found in mushrooms. The larval, or maggot, stage lasts about 10–14 days, depending on environmental conditions. The maggot crawls into the soil or manure and transforms into a pupa. This stage lasts from 4–7 days, after which the fly emerges. The fly is ready to lay eggs within a few hours. The complete life cycle ranges from 18–26 days, depending upon temperature conditions.

FIGURE 81.—Fungus gnat larva.

MUSHROOM MITES.—There are four species of mites (Figure 82) which are pests of mushrooms. The mite most commonly found among the cultivated type of mushroom is *Tyroglyphus lintneri*. These mites damage the mushrooms by eating holes in the caps and stems. They also occur in the spawn and if extremely numerous may consume all the spawn. Since mites are almost microscopic in size they often are overlooked. Unless they occur in large numbers the damage caused may be slight.

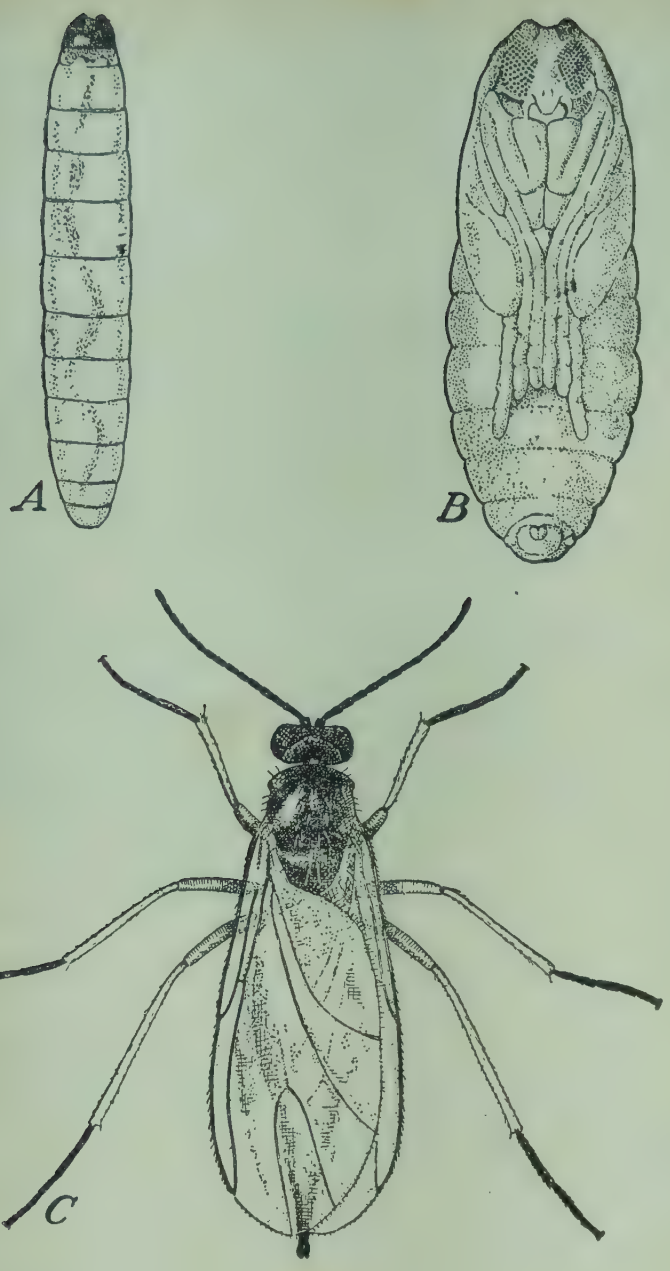
SPRINGTAILS.—These are small, gray, black or brown insects, hardly ever more than 1 mm. in length (Figure 82). Most of them inhabit wild mushrooms of various types but frequently may be found in cultivated types of mushrooms. They enter the mushroom houses in compost piles used in filling the mushroom beds.

The life history of these insects is simple. The eggs hatch in about 10 days into nymphs (insects resembling adults). These nymphs mature in a short time. Damage to mushrooms begins almost as soon as the egg hatches. In cultivated mushrooms these insects damage the spawn material and destroy most of the mycelium, often times making it necessary to replant fresh material.

SOWBUGS.—These are oval-shaped, gray to grayish-brown creatures (Figure 82). They are not true insects but are crustaceans related to the lobster or crab. They often gain entrance to mushroom-growing houses through the manure used for the mushrooms. They break down and destroy the bed-manure and chew holes in the small mushrooms.

SLUGS.—These are soft-bodied snails without shells and often damage mushrooms by chewing holes in the caps or stems.

FLAT-FOOTED FLIES.—The adult fly is quite similar to the house fly but much smaller in size. The larvae (or maggots) and puparia are much alike. The maggots are small, flattened, and cream-colored, with



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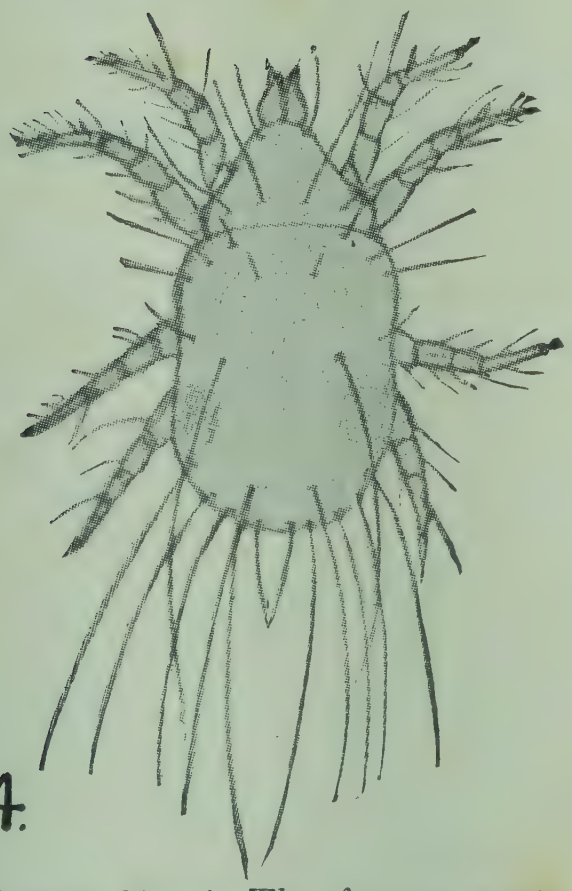

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FIGURE 82.—1. The fungus gnat: *A*, Larva; *B*, Pupa; *C*, Adult. 2. Mushroom damage caused by the fungus gnat larvae. 3. Sowbug (*Porcellio laevis*). 4. Mushroom mite (*Tyroglyphus longior Gerv.*). 5. Springtail (*Achorutes armatus Nic.*). (From: 1—Circ. 457, U. S. Dept. Agric.; 2 to 5—Pennsylvania Agric. Expt. Sta. Bull. 419.)

segmented spiniferous processes on the sides of all the segments except the first, second, and last. They live in the fleshy portions of mushrooms, and other types of fungi upon which they feed. The cream-colored maggots are known to inhabit mushrooms on the West Coast.

2. Description of Slides of Authentic Filth Material.

Slides of Whole Insects, Insect Parts, Rodent Filth, and Materials of Common Occurrence that Sometimes are Confused with Filth. In addition to the foregoing descriptive material certain permanent filth exhibits have been prepared for use in the training of analysts and for reference material. The whole insect collection includes many of the common storage pests in both their adult and larval forms, some of the field pests, and some general contaminants such as ants and flies. Two insects have been dissected and some of their parts mounted in slides: the confused flour beetle is used as an example of the small storage beetle type; the housefly as an example of filth vectors. In addition, some life history exhibits in the nature of pupae and eggs have been prepared along with some insect excreta mounts.

The slides are divided into two series both of which are labeled "I" for "insect" although some rodent and other filth is included. One series, "I–R," contains the whole insects and large particles mounted in a thick cell formed by means of a brass "ring."[3] The other series with the coverglass mounted flat to the slide has the individual slide number prefixed only by an "I."

I–R–1. Small eyed flour beetle: See key to storage insects I, B, 1, b, (B), (2), (b), A′, 2′; also note the regular square pattern on the elytra.

I–R–2. Lesser grain borer: See key to storage insects I, B, 1, b, (B), (1), (a). This is a typical borer. The roughened surface is particularly noticeable on the dorsal surface of the prothorax. Note also the spines on the legs especially those on the tibia of the first two pair.

I–R–3. Flat grain beetle: See key to storage insects I, B, 1, b, (B), (2), (b), A′, 1′, a′. The relatively long antennae on this small beetle are quite characteristic. Note also the parallel longitudinal lines in the elytra.

I–R–4. Rice weevil: See granary weevil; key to storage insects I, A, 1, a. This and the granary weevil (I–R–8) are typical weevils with elongate snouts terminating in the mouth-parts. Antennae appear as two horn-like elbowed projections.

I–R–5. Sawtoothed grain beetle: See key to storage insects I, B, 1, b, (B), (2), (a). The toothed thorax is a pronounced characteristic.

I–R–6. Cadelle: See I–R–16; key to storage insects I, B, 2, b, (B). This beetle is readily distinguished by its size, color and shape; by the "beaded" edge on the lateral margins of the prothorax and the projections of the prothorax into a lateral horn on each side of the head.

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[3] All rings prepared by A. G. Sterling, instrument maker, U. S. Food and Drug Administration.

I–R–7. Rust red flour beetle: See I–R–15; key to storage insects I, B, 1, b, (B), (2), (b), B′, 2′, b′. This beetle and the confused flour beetle are similar in appearance. Both species are common stored-food pests. They may be found either separately or together.

I–R–8. Granary weevil: See key to storage insects I, A, 1, b; comments on the rice weevil. Note in both species the spade-like, toothed tibia.

I–R–9. Indian meal moth larva: See key to storage insects II, B, 2, a. This is a typical caterpillar pest of stored foods. Like many other caterpillars it has six thoracic or true feet terminating in a claw and eight abdominal pseudopodia and a pair of anal pseudopodia terminating in a row of incurved hooks.

I–R–10. Rust red flour beetle larva: See I–R–7. No attempt should be made to distinguish between this larva and that of the confused flour beetle, I–R–13. Both are common in infested foods and both have conspicuous forked terminal spines which are readily recognized even in comminuted products.

I–R–11. Small eyed flour beetle larva: See I–R–1. The comparatively large antenna and dark pigmented eye spot are characteristic. Note also the two small flap-like ventral anal appendages and the small bifurcate dorsal terminal spines which are much smaller than those of the *Tribolium* spp. (I–R–10 and 13).

I–R–12. Cowpea curculio larva: This larva and larvae of some other coleoptera are stout, usually curved, legless grubs. This slide represents one of this type obtained from succulent cowpeas in Georgia. As larvae many of the species are identified with difficulty even by experts.

I–R–13. Confused flour beetle larva: See I–R–10.

I–R–14. Cabbage aphids: These aphids are similar in appearance to most aphids infesting succulent green vegetables. They have delicate appendages and plump bodies with a pair of latero-dorsal tubes or cone-shaped cornicles on the abdomen. Note the peculiar jointed antennae with an abrupt reduction in size of the terminal segment; the elongate cone-shaped mouth-parts, and the simple tarsal claws. When wings are present the venation is simple, but most forms are wingless.

I–R–15. Confused flour beetle: See I–R–7; key to storage insects I, B, 1, b, (B), (2), (b), B′, 2′, a′.

I–R–16. Cadelle larvae skin: See I–R–6. This is not a true cast skin but a larva split along the mid ventral line and with the viscera removed to give a clearer picture of the skin itself. This larva is as readily recognized as the adult. Note the darkly pigmented elongate head capsule, brown prothoracic shield and paired dorsal pigment spots on the second and third segments of the thorax. At the posterior end is a tough brown stoutly constructed fork.

I–R–17. Housefly: See I–R–23–28–34–35; I–18–19–20–21–22; key to storage insects V.A. Certain features of the housefly are typical of most flies. For example, note the slender hairy legs (I–22) which are characteristic of many insects that do most of their traveling by flying. There is one pair of wings, the second pair being represented by a pair of knobbed organs called "halteres." The wings are veined and have scales and spines (I–20). The halteres (I–21 and Figure 83) are located posterior to the attachment of the true wings. The seta-covered body is also characteristic of many flies. Note on slide I–19 that the setae have rounded bases and arise from pits in the cuticle. This fact is of considerable importance in the determination of insect fragments and in distinguishing between setae and plant hairs.

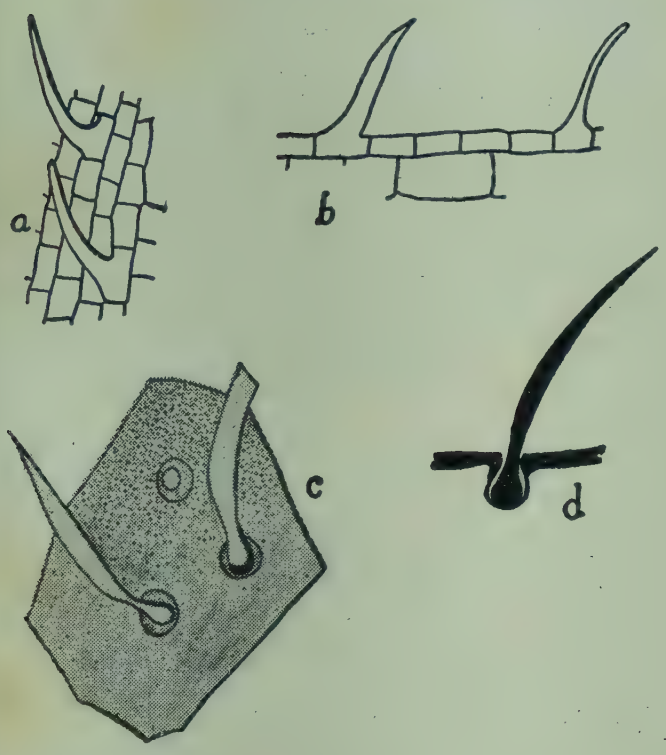

FIGURE 84.—Articulation of plant and insect hairs. *A* and *B*, The plant hairs arising as outgrowths from the epithelial cells; *C* and *D*, Insect setae arising from pit.

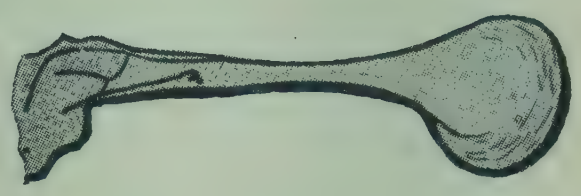

FIGURE 83.—Halter.

Note in Figure 84 that the bulb-like base from which the insect setae arise fits with a ball and socket effect into a pit in the cuticula. Insect scales (slide I–17) are modified setae and are similarly articulated to the insect body. Note also the slides of various insect larvae and their setae. These slides of insect larvae especially I–R–16 and also I–19 show the lack of layers observable with the magnification (up to 200x) ordinarily used in food and drug insect contamination work. Along with the fleshy sponge-like tarsal pad and proboscis these hairs are responsible for the carrying of filth by flies. Slide I–18 is a view of the proboscis used for the softening and lapping up of liquid food. The antenna ((I–R–34) is a club-shaped organ with a spine-like arista arising from it. The antennae fit into a recess in the front of the head. The large compound eyes take up most of the head surface and are composed of innumerable tiny facets (I–R–35).

I–R–18. Purple scale: The scale insect itself is a minute form, with scarcely any more than rudiments of appendages, which lives protected beneath the scale or covering which it secretes. Many of the scale coverings are somewhat clam or oyster-shell shaped.

I–R–19. Cigarette beetle: See key to storage insects I, B, 1, a, (B). This is a somewhat hump-backed stout beetle with rather inconspicuous antennae arising from recesses in front of and below the eyes.

I–R–20. Confused flour beetle elytra: This is one of a series including I–R–21, confused flour beetle abdomen; I–R–27, confused flour beetle pupae; I–R–30, prothorax; I–R–36, egg; I–10, antenna; I–11, maxilla and labium; I–12, labrum; I–13, mandible; I–14, scutellum; I–15, hind wing; and I–16, legs, which is designed to give a picture of the type of material encountered when a typical cereal-infesting beetle is comminuted. Most of the important appendages are represented. Details of some of the body structures can be obtained from the mounts of whole insects. When the insects are broken during grinding, the pieces may assume the most bizarre shapes and it is only by recognizing the fragment as a part of the whole insect that a satisfactory count of fragments can be made. The most difficult distinctions which must be made are between insect fragments and certain plant tissues such as leafy material, bran, chaff, and seed coats in general. So far as this discussion is concerned all plant tissues can be considered to be composed of cellular material with definite cell walls which are readily visible without the use of stains. (Figure 85). Figure 86*A* shows the under epidermis of a leaf with the characteristic stomata composed of a 2-celled bivalve arrangement around a central pore. None of these characters is present in insect cuticle. Figure 86*B* shows the lenticles on bark which in crude drug bark should not be confused with scale insects.

The insect cuticle presents a quite different appearance. As shown in Figure 87 the overlying cuticula is non-cellular and is secreted by the underlying epidermis. While the epidermis is cellular, the cell walls, like those of many animal cells, are not visible without being subjected to special treatment. Moreover in fragments encountered in filth analyses the only discernible part is the non-cellular cuticula. This fact is basic.

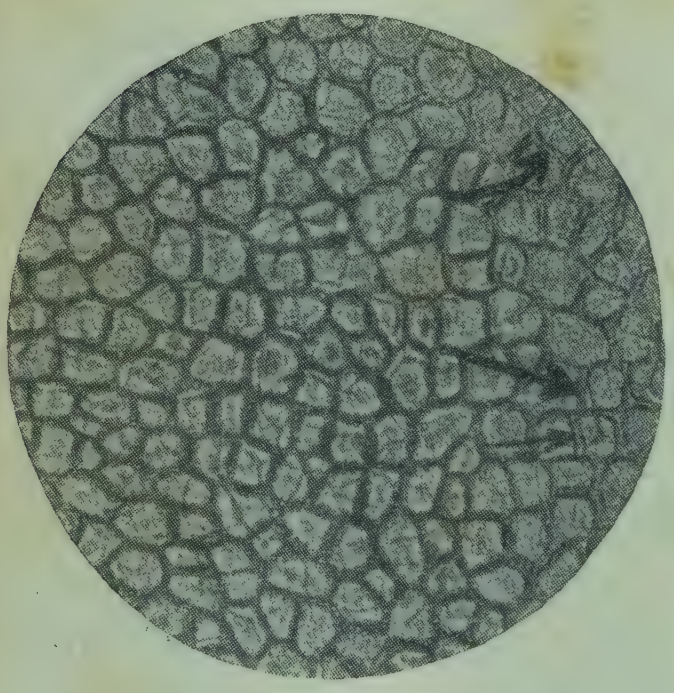

FIGURE 85.—Tomato epidermis. Note the unevenly arranged cell walls of more dense material. The walls of adjacent cells are separated by the so-called middle lamella, visible (arrows) as a thin line.

Insect fragments also have certain "landmarks" which identify them as insect fragments. Note in slide I–R–20 the regular "moulded" or "rolled" edge and the complex symmetry of the point of articulation with the rest of the body. Similarly, segments of the abdomen (Slide I–R–21) are evenly joined together along a thickened line and the general symmetrical outline is quite apparent. Note especially that where the abdomen and thorax join there are paired recesses into which the coxae of the hind legs fit. This type of structure is of quite common

occurrence for a similar rounded cavity occurs at the articulation of the legs, antennae, mandibles, and maxillae with the body. The thickened sutures or overlapping of adjacent abdominal segments are also characteristic. The only landmark, on an otherwise questionable insect fragment, may be a suture. Note also the cuticular covering of the confused flour beetle pupa (Slide I–R–27) which is very thin and pale in color but without cellular structure; it is composed of a series of rather translucent plates.

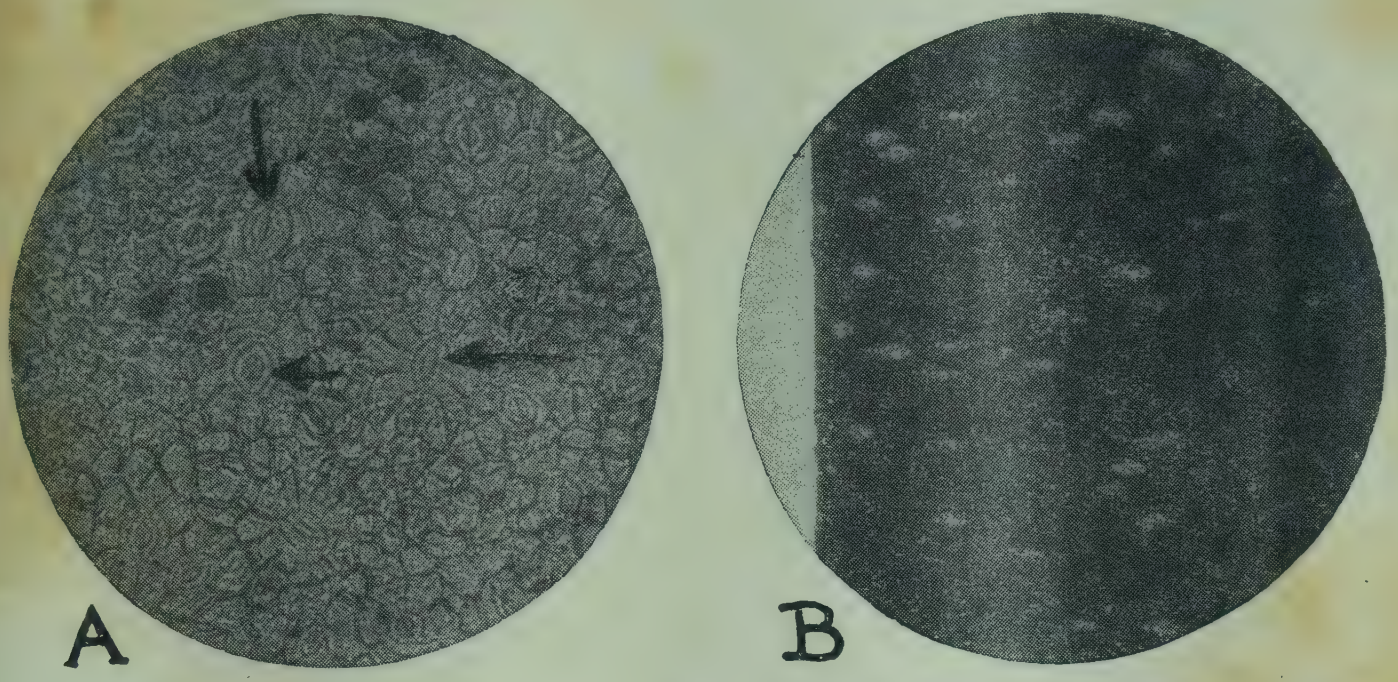

FIGURE 86.—*A*, Under epidermis of leaf (arrows indicate stomata); *B*, Lenticles on bark.

Slides I–10, –11, –12, –13, –15, and –16 show confused flour beetle appendages. These parts are similar to comparable structures on many storage beetles and should be studied with considerable detail. Note especially I–11 and –12 of the maxilla, labium, and labrum. See Figure 49 for the relationship of these parts. The labrum is the flap-like upper lip which retains a remnant of its ancestral bilateral origin in the form of a rudimentary cleft. It is attached to the also flap-like clypeus. Both of these structures are frequently encountered in insect-infested products. The mandible is more universally recognized, but the more complicated maxillae with its palpi (fingers) warrant detailed study. In these slides the labium or lower lip remains attached to the maxillae in its natural position. The labium is somewhat similar to the labrum except that it is much smaller and has a pair of palpi. I–14. The first pair of wings (elytra) are attached to the scutellum which is a small (in the storage beetles), roughly triangular, dorsal plate located just posterior to the shield-like prothorax. In the true bugs (Figure 61) this scutellum is large and prominent.

I–R–21. Confused flour beetle abdomen: See I–R–20.

I–R–22. German roach (Croton bug, water bug): Key to storage insects III, A. This is one of the more common cockroaches. It is readily identified by its pale color and dark markings on the pronotum. The other cockroaches are somewhat similar in appearance to this species but many attain a length of several inches.

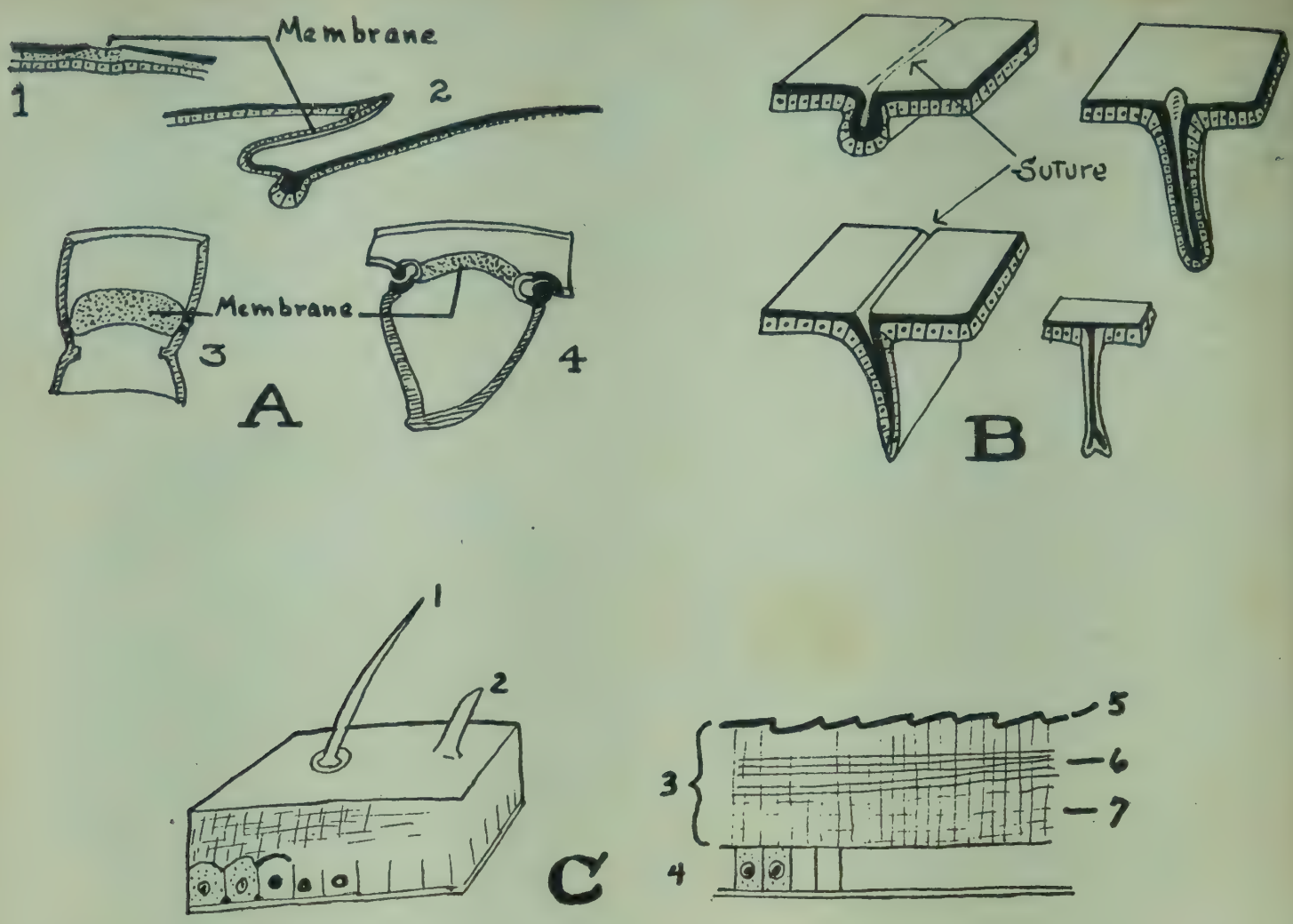

FIGURE 87.—The integument, diagrammatic sections. *A*, Sutures, joints, articulations: 1, simple membranous suture; 2, membrane between two body segments; 3, leg joint, dicondylic; 4, mandible, dicondylic. *B*, Types of apodemes. *C*, Stratified structure: 1, seta; 2, spine; 3, cuticula; 4, epidermis; 5, epicuticula; 6, exocuticula; 7, endocuticula. (Cellular structure not visible unless stains are used.) (From Snodgrass, Principles of Insect Morphology. By permisson of McGraw-Hill Book Co.)

I–R–23. Housefly maggot: See I–R–17. This is a typical fly larva or maggot. Note that it is legless, tapered anteriorly, has two breathing pores or spiracles at the posterior end. There are paired darkly pigmented mouthhooks at the anterior end which are used by the living maggot in ingesting food. These mouthhooks are important in filth work because a pulping or straining of a food product containing whole maggots may either destroy or remove other visible evidence of their presence while the small but tough mouthhooks may still be found. Also note the presence of small pointed spines arranged in bands and in isolated patches on the cuticula. These are the locomotor spines (Figure 88). These spines are a part of the cuticle and are not to be confused with the tactile setae. Spines of a similar histological origin occur in other adults and larvae, and pupae, but the characteristic banding which usually is disposed circularly around or on the ventral surface of the maggot is a character which can be used as an aid to the identification of maggot skin.

I–R–24. Blueberry maggot: See I–R–23. This is a smaller maggot than that of the housefly but is similar in appearance to that maggot and to many maggots infesting fruits and berries.

I–R–25. Broad horned flour beetle (male): Key to storage insects I, B, 1, b, (A), (1). The huge forceps-like mandibles of the male and the shape of the head are very characteristic. Note the lateral leaf-like extensions of the head and the anterior-dorsal protuberances in the male. The female head (not shown) has a large flat covering which extends over the anterior margin.

I–R–26. Drugstore beetle: Key to storage insects I, B, 1, a, (A). This beetle received its common name because it was a common pest of crude drugs.

I–R–27. Confused flour beetle pupae: See I–R–20.

I–R–28. Housefly pupae: See I–R–17.

I–R–29. Mediterranean flour moth: Key to storage insects II, B, 2, c, (B). The adult moth does not feed but the caterpillars of this and the other *Ephestia* species are destructive pests of stored foods.

I–R–30. Confused flour beetle prothorax: See I–R–21. This most anterior thoracic segment with the conspicuous dorsal shield is one of the most striking features of many insect groups. The prothorax of this beetle is similar to those of most of the reddish brown storage beetles. When fragmented it may appear quite similar to some weed seed coats. The line of fusion between the dorsal and ventral sclerites (plates) plainly is visible as are the thickened edges of the intersegmental sutures.

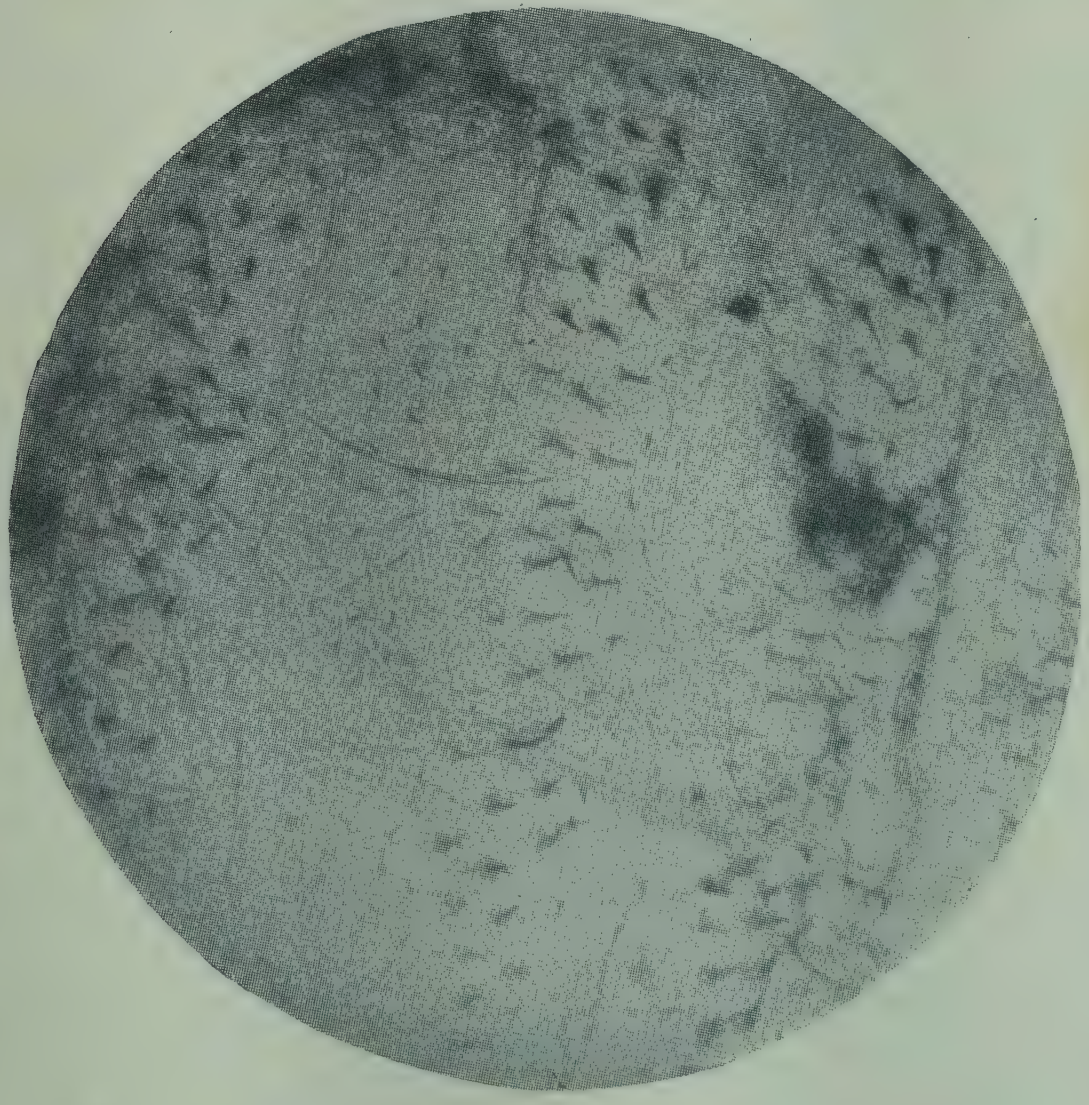

FIGURE 88.—Locomotor spines on housefly maggot.

I–R–31. Excreta Indian Meal Moth Caterpillar: The food-infesting caterpillars in general are larger than the larvae of food infesting beetles (exception: the cadelle and mealworms of coleoptera) and their excreta is large and chunky. This excreta is dark because the larvae were feeding on dried prunes. On more pale foods the pellets would be lighter in color. Note slides I–7 and I–27, excreta of the sawtoothed grain beetle and confused flour beetle. No attempt should be made to identify insect species by the excreta they have deposited in the food but insect excreta can be recognized as such from its distinctive rounded appearance. It usually has a laminated or packed or heterogeneous appearance (see Figure 89) with

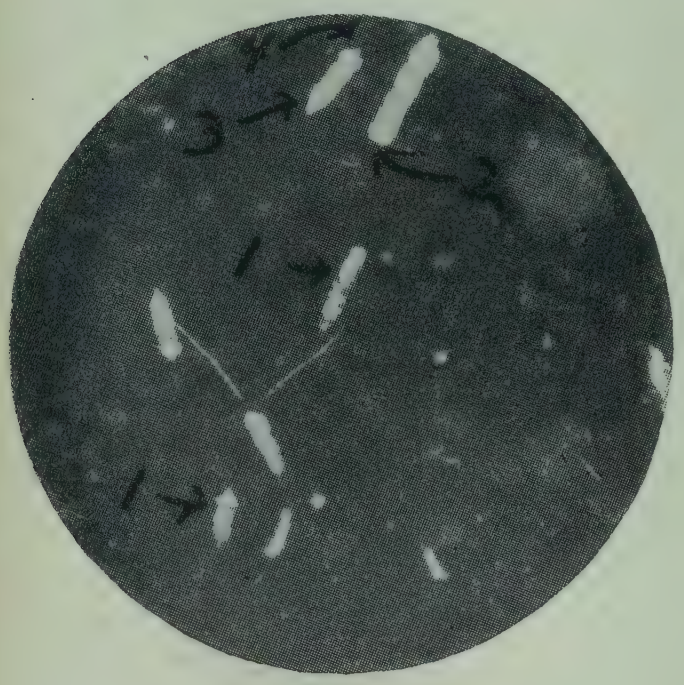

FIGURE 89.—Excreta sawtoothed grain beetle. 1, Laminated appearance; 2, flat "cut-off" end; 3, tapering end; 4, nib-like projection. (Preparation cleared in clove oil and photographed by reflected light.)

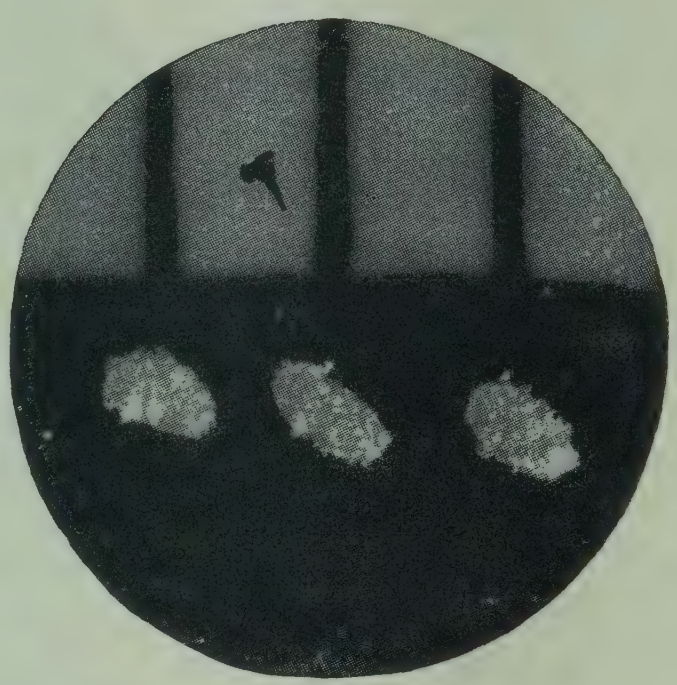

FIGURE 90.—Confused flour beetle egg from flour. (Lines are on a millimeter scale.)

dense masses separated by more hyaline areas as if consecutive meals were piled one behind the other. The ends may terminate squarely and abruptly but more often one or both of the ends are tapering or with a nib-like projection.

I–R–32. Allegheney Mound Building Ant: Key to storage insects IV, A, 1. This ant is included in the collection simply as a representative of the group. Its size and color make it particularly useful for study. Note the massive head, elbowed antennae, the petiole or stalk between thorax and abdomen, and its naked appearance.

I–R–33. Pavement Ant: See I–R–32. This is the common small black ant frequently found under pavement blocks in the Eastern United States and at times found in buildings.

I–R–34. See I–R–17.

I–R–35. See I–R–17.

I–R–36. Confused flour beetle egg: These eggs may be sifted out of flour on a 5xx bolting cloth. They are glistening white but particles of food readily adhere to them. (See Figure 90). This egg is typical of many storage beetles. Some are larger, some smaller, some more elongate, and some more robust. Eggs extracted from foods or dried out may be shriveled beyond recognition.

I–R–37. Dried fruit beetle: See I–R–38; key to storage insects I, B, 2, c, (B), (1), (a). This is a cosmopolitan species distributed on dried fruit over the world. Both adults and larvae are readily distinguished from other small food-infesting beetles. Note the hairy and pitted pronotum and the bulging eyes of the adult in addition to the characters noted in the key. Beyond the extension of the short elytra the abdomen is hairy.

I–R–38. Dried fruit beetle larvae: See I–R–37. The two pairs of tubercles at the posterior end are diagnostic. Note also that the head is separated from the thorax by more of a neck-like constriction than is present in most larvae.

I–R–39. W. I. I. R. usually consists of soil particles such as compounds of iron and other metals, and sand which is essentially fine grains of quartz.

I–R–40. Ground glass is singly refracting and does not show any display of colors when examined with crossed nicols. The particles are irregular and angular and generally possess conchoidal fracture.

I–R–41. A. I. I. R. consists essentially of fine grains of quartz which are doubly refracting with either rounded or less angular edges than ground glass. Small amounts of other minerals may be present.

I–1. Mushroom mite: Key to storage insects VI. The general size, shape, presence of elongate hairs, pointed rostrum, simple legs are characteristic of most food-infesting mites. Cereal mites, sugar mites, mites in dried fruits, etc. bear a striking resemblance to this species.

I–2. Rat excreta fragment with hair: The heterogeneous composition, including typical rat hairs, is plainly visible. Note that without the hair the dark pellet fragment resembles a bit of moist soil.

I–3. Rat guard hairs (See Section III Rodent Contamination.)

I–4. Rat fur hairs (See Section III Rodent Contamination.)

I–5. Mouse fur hairs (See Section III Rodent Contamination.)

I–6. Cat fur hairs (See Section III Rodent Contamination.)

I–7. Excreta sawtoothed grain beetle on peanut: See Slide I–R–31.

I–8. Cat guard hairs (See Section III Rodent Contamination.)

I–9. Cow hairs, back and abdomen (See Section III Rodent Contamination.)

I–10. Confused flour beetle antenna: See I–R–20.

I–11. Confused flour beetle maxilla and labium: See I–R–20.

I–12. Confused flour beetle labrum: See I–R–20.

I–13. Confused flour beetle mandible: See I–R–20.

I–14. Confused flour beetle scutellum: See I–R–20.

I–15. Confused flour beetle hind wing: In beetles the forewing is horny or leathery and, when present, the hind wings are thin and adapted for flight although many stored foods pests seldom fly and the hind wings are not used. Some beetles have only rudimentary hind wings and others have the elytra fastened down against the abdomen so that the hind wings are quite useless. The veination of this wing is relatively simple and it would not be mistaken for that of a fly (I–20) which has a covering of scales and setae and more complex pattern of veins. The aphids (I–34) have a distinctive simple pattern consisting of a relatively wide heavy vein across the leading or anterior edge of the wing with a few laterals arising from it.

I–16. Confused flour beetle legs: See I–R–20.

I–17. Scales of the Mediterranean flour moth: The coloration and pubescence of moths and butterflies is formed by the presence of thousands of these overlapping leaf-like scales. Similar scales occur in other groups, e.g. silverfish (Order Thysanura). These scales are commonly found in cereal products made from infested grain.

I–18. Housefly proboscis: See I–R–17.

I–19. Housefly abdomen fragment: See I–R–17.

I–20. Housefly wing: See I–R–17.

I–21. Housefly halter: See I–R–17.

I–22. Housefly leg: See I–R–17.

I–23. Cotton fibers: See also I–24–25–26–28–29–30. Plant and animal fibers often are present in a filth examination. Cotton fibers are readily distinguished from animal and insect hairs by the fact that they are flattened bands with a significant twist. Linen fibers (I–24) can be determined by their smooth rounded outline and characteristic transverse lines. I–25 shows wool with overlapping scales and the type of medulla separates wool and dog hairs (I–28) from rodent hairs. Jute fibers (I–26) commonly are found in foods as are feather barbules (Slide I–29) (Figure 91 *B*). The joint-like swellings sometimes contain pigmented areas and the barbules then bear a superficial resemblance to rodent or cat fur hairs. They also resemble certain jointed insect setae such as the hairs from dermestid larvae (Figure 91 *A*) which are almost antenna-like. Other plant fibers, e.g. dandelion pappus Slide I–30

may be confused with the insect hairs bearing sharp spines (Figure 92) and multicellular plant hairs (Figure 93) may appear much like aphis or mite legs (I–32) when viewed with the low power Greenough-type microscope. When these types of slender fibers, hairs, and setae are studied together at 100–200x the distinctions are, at once, apparent and they can then be recognized even at 25–40x. The plant fibers never are truly jointed. They have no distinct segments that articulate with adjacent segments. The plant fibers show the pronounced cell walls which are characteristic of most plant material, and when examined critically even the apparent segmentation of the feather barbules can be distinguished as swellings and constrictions rather than

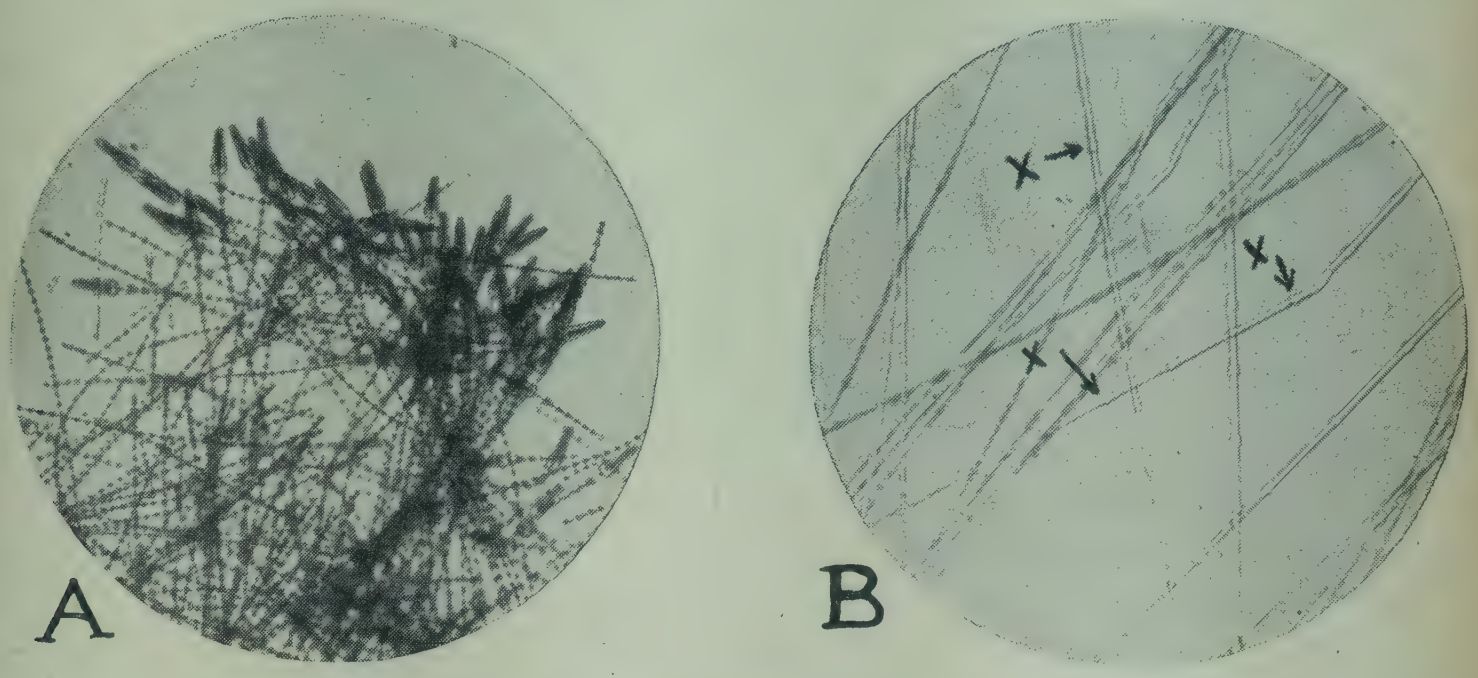

FIGURE 91.—*A*, Compound insect hairs from dermestid larvae; *B*, Feather barbules, joint-like swellings indicated by "x."

true segmentation. Note that honeybees and other insects may have plumed setae which often resemble the arista on a fly antenna (see I–R–34)) although they are more densely clothed with branches than is the arista and the branches may not extend as far from the main shaft. The pitted articulation of setae (see I–19) is present on all of the plumed or "compound" insect hairs.

I–24. Linen fibers: See I–23.

I–25. Wool fibers: See I–23.

I–26. Jute fibers: See I–23.

I–27. Confused flour beetle excreta: See I–R–31.

I–28. Dog hairs: See I–23.

I–29. Feather barbules: See I–23.

I–30. Dandelion pappus: See I–23.

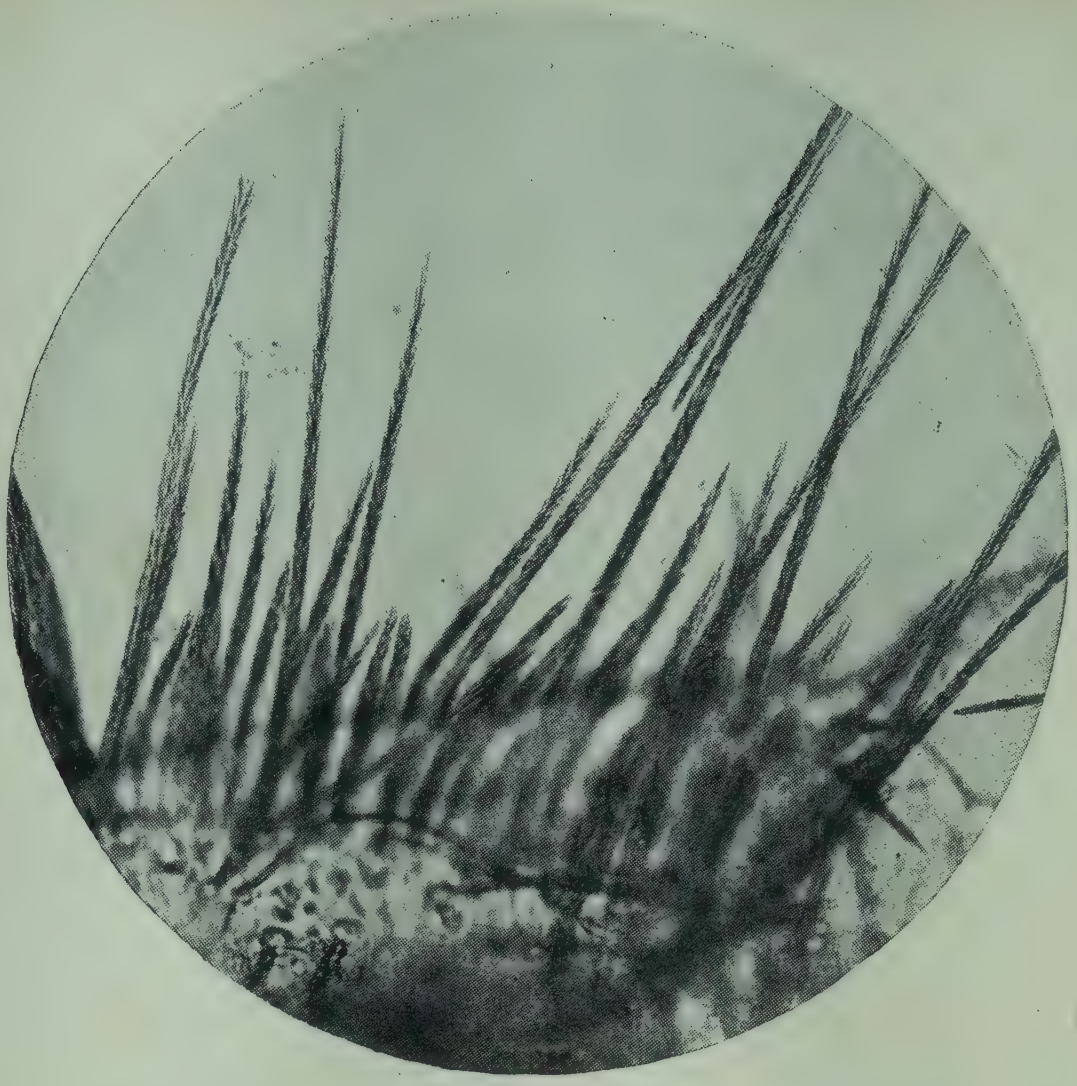

FIGURE 92.—Spined hairs from dermestid larvae.

FIGURE 93.—Multicellular plant hairs.

I–31. Tannin cells: These smooth amber-colored cells occur in many plants. They are particularly abundant in the septa of pecans and when scattered over broken surfaces of the nutmeats they look to the unaided eye about like insect excreta pellets (see I–R–31) from which they can be distinguished by their homogeneous smooth, rounded, glistening appearance. They turn dark blue-black in soluble ferric salts.

I–32. Aphis legs: These rather simple, smooth, naked, slender, fragile legs are found in considerable numbers in aphid-infested foods.

I–33. Aphis antenna: These antennae have a peculiar "stepped-in" structure on the last segment where the segment abruptly becomes about half as wide as it was at the basal end of the segment. On the long segment following the shorter barrel-like ones, small ringed thickenings usually can be seen.

I–34. Aphis wings: See I–15.

XII. DIRT

For the purpose of this discussion dirt is considered to include, in addition to sand and soil, any substance such as excrement, dust, etc. which pollutes a thing or makes it filthy. Soil, in general, is the loose surface material of the earth containing sand, organic matter, and soluble salts. Any one or all of these substances may be encountered in the examination of food products. While the above definition of dirt includes excrement, the latter is considered to be of a more repulsive nature than the dirt which usually contaminates food products. In fact, a food product that is contaminated with insect excreta, while it may be dirty, is classed as insect-infested. Similarly, a product contaminated with rodent excreta is reported as rodent-infested. Other excreta, if it can be identified, is classified according to its animal source. The following discussion is arranged according to the different food products commonly affected.

Candy. While macroscopic dirt on candy is not particularly common, it is encountered occasionally. No clear-cut standard has been established for judging dirty candy and it is necessary, for a basis of judgment, to rely upon the analyst's own idea of what is dirty or repulsive.

Dried Cut Fruits. A piece of dried fruit is classified as dirty if it is contaminated with foreign material such as sand, grit, mud, dust or nondescript dirt, giving it a dirty, smudgy appearance. The ability to judge whether or not a piece of fruit is sufficiently dirty to be classed as a reject comes with training and experience of the analyst. The pieces of fruit should be examined individually and thoroughly with good illumination.

Dried Figs. What has been said above about cut dried fruits will also apply to dried figs.

Nuts. The problem of dirt in shelled nuts is particularly important in the case of shelled peanuts. Because they develop underground, peanuts in the shell are naturally contaminated with dirt. During the process of shelling or, because of accidental damage to the shells, a certain amount of this dirt is transferred to the exposed surfaces of the split kernels. This dirt usually consists of sand and soil particles. Some lots of shelled peanuts contain appreciable numbers of peanuts that are only slightly dirty; these are not considered as rejects. Frequently the discoloration of these nuts is caused primarily by the presence of fine particles of testa (peanut skin) embedded in the exposed surfaces. This condition, while unsightly, is not particularly objectionable and these particles do not add to the weight of the water-insoluble inorganic residue in peanut butter.

Nut meats, other than peanuts, do not normally come in contact with soil and hence the standard for such nuts, based on good commercial practice, is higher.

Residues of dirt which for the most part are water-insoluble are frequently encountered in the examination of a number of food products. These residues may consist of any or all of the following: Rodent excreta—identified by the presence of embedded rodent hairs; insect excreta; sand; soil; glass; metal; and nondescript dirt. They should be examined under the low-power microscope for identity and, when it is called for in the method, a quantitative determination should be made.

XIII. MOLDS IN FOOD PRODUCTS

The fungi, quite commonly called molds, comprise a large group of chlorophyll-free thallophytic organisms whose structure is basically mycelial in habit. These organisms are of great economic importance as plant pathogens which cause serious crop losses and as a cause of decomposition and spoilage of stored food products. Although many types of fungi have been utilized for the benefit of man, it is chiefly in their role as destructive parasites and saprophytes on the food and drug supply that we are concerned.

Fungi are plants whose basic structure, the mycelium, for most organisms in this group, consists of branched, microscopic cellular filaments. A single filament of the mycelium is called a hypha. In most species, the hyphae are richly branched, producing a mass of threads having the appearance of a cottony mass. The term mold is usually associated with this effect. This mycelial growth comprises the vegetative form of the fungus and from it arise the asexual reproductive bodies, called spores. The method and type of spore production vary in the different groups and this forms a basis for the classification of fungi.

Because of their lack of chlorophyll, fungi are unable to manufacture their own food and hence must secure their nutriment from other host organisms as PARASITES, or from the dead tissues and waste products of organisms as SAPROPHYTES. Although other types of nutrition exist among the fungi, their modes of existence as saprophytes or parasites account for their role as plant pathogens and as agents in the cause of rot and decomposition.

A. Taxonomic Characteristics of the Fungi

The classification of the forms of fungi is based on the reproductive bodies produced. These may be (1) asexual or vegetative bodies which are produced directly on the fungus mycelium or (2) they may be sexual structures in which there is a fusion of nuclear material which results in the formation of special types of spores. In the PHYCOMYCETES, ASCOMYCETES, and BASIDIOMYCETES both asexual and sexual types of spores are produced. The class, FUNGI IMPERFECTI, include those forms of fungi in which only the asexual type of spores has been found, hence these forms are known as the IMPERFECTS.

The fungi are grouped into the following 6 large classes:

1. Schizomycetes—Bacteria
2. Myxomycetes—Slime Molds
3. Phycomycetes—Alga-like fungi
4. Ascomycetes—Sac fungi
5. Basidiomycetes—Basidium fungi
6. Fungi Imperfecti—Imperfects

B. Characteristics and Descriptions of the Classes of Fungi

1. Schizomycetes—Bacteria.

These forms are microscopic, unicellular organisms. They consist essentially of 3 forms: (1) cocci—spherical shape; (2) bacilli—straight rods, and (3) spirilli—curved rods.

Reproduction is chiefly by fission or direct division of a cell. A few forms are capable of producing from one to several spores. The spore-bearing types are rod forms and spores are never found in the spirilli and cocci. Though bacteria were first discovered by Loewenhoek in 1683 they were not known as plant disease organisms until 1879 when they were shown by Burrill to be of etiological significance in pear blight. Bacterial diseases produce several distinct types of symptoms in plants such as (a) soft rots; (b) canker; (c) leaf-spot; (d) galls; and (e) vascular wilt.

The following table includes some of the diseases caused by bacteria:

HOST	COMMON NAME	SCIENTIFIC NAME
Tomato	Bacterial canker	*Phytomonas michiganensis.*
Tomato	Bacterial speck	*Bacterium punctulans.*
Tomato	Bacterial spot	*Pseudomonas vesicatoria.*
Tomato	Fruit rot	*Erwinia aroideae.*
Turnips, Beets, Carrots, Cabbage, Rutabagas	Soft rot	*Erwinia carotovora.*
Beans	Blight	*Phytomonas phaseoli.*
Tomato, Potato, Egg Plant	Brown rot	*Phytomonas solanacearum.*
Peach, Plum	Black spot	*Phytomonas pruni.*
Apple	Blister spot	*Phytomonas populans.*
Citrus fruits	Blast and Black pit	*Phytomonas syringae.*
Cauliflower	Spot disease	*Phytomonas maculicolum.*

2. Myxomycetes—Slime Molds.

These organisms include the simplest types of fungi. The outstanding characteristic is that the vegetative condition consists either of distinct amoeboid cells or of a mass of naked protoplasm, called the plasmodium. These molds have little importance as rot-producing organisms in foods or food products. Only one form, *Plasmodiophora brassicae,* which causes the clubroot of cabbage and related plants, is of economic importance as a plant disease organism.

3. Phycomycetes—Alga-like Fungi.

The chief characteristic of these fungi is the production of non-septate hyphae. There are some 800 species which vary in form from those with a simple, poorly developed mycelium to types consisting of a richly branched mycelium. Reproduction is by means of either (1)

sexual spores, formed by the union of gametes, or by (2) asexual spores produced by the vegetative mycelium.

There are two forms which are commonly encountered as rot-producing fungi. These are *Mucor* and *Rhizopus*. They cause spoilage of various kinds of foods and food products, and are common in both field and storage conditions. Many fresh fruits and vegetables become contaminated with these organisms during the process of marketing. Rough

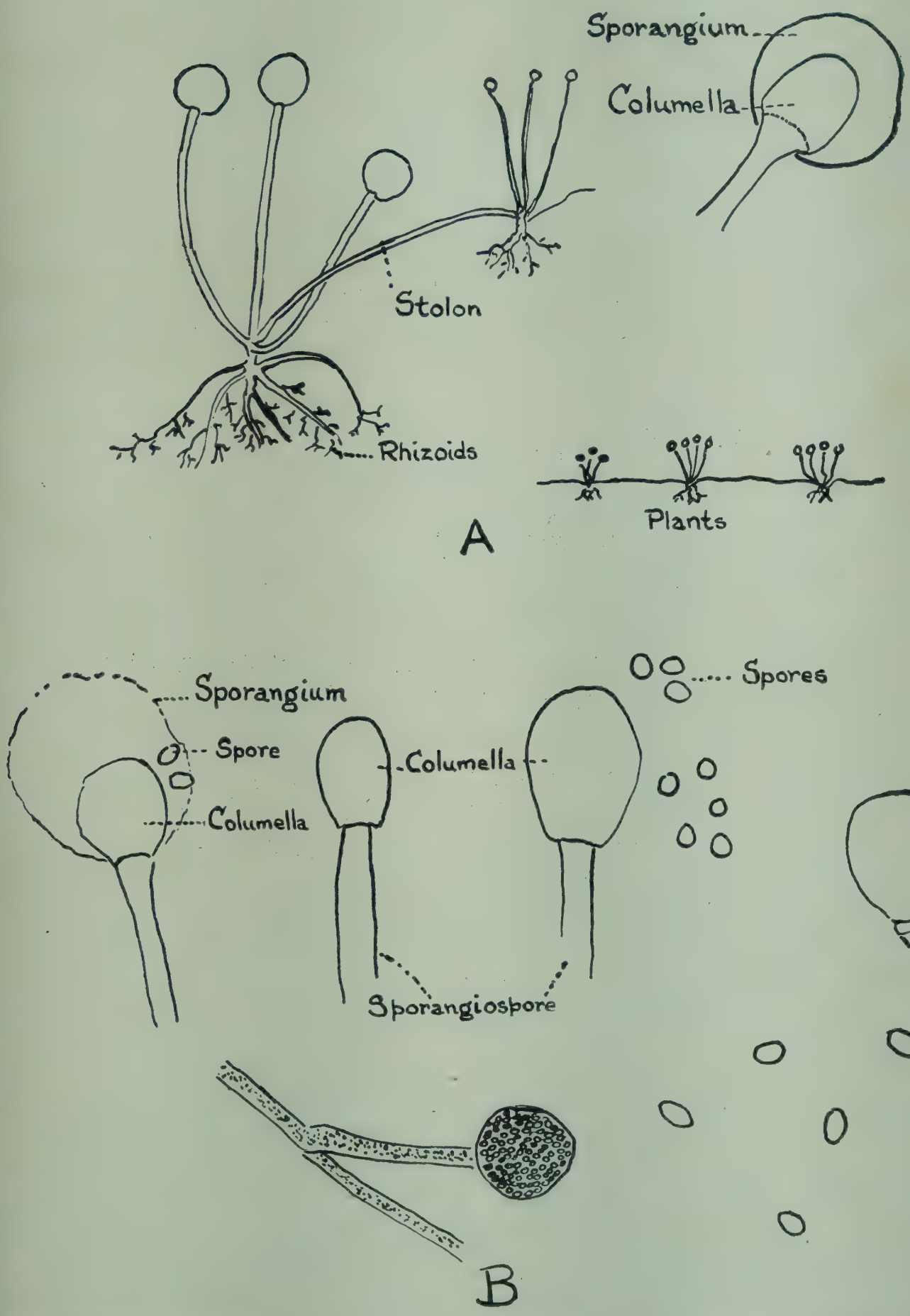

FIGURE 94.—Common black molds. *A, Rhizopus; B, Mucor,* from tomato. (*A* from Fitzpatrick, The Lower Fungi-Phycomycetes. Plants from Lutman, Microbiology. By permission of McGraw-Hill Book Co.)

handling which causes bruises and cracks in fruits and vegetables serves as a means of entrance for these fungi. Under favorable climatic conditions they develop rapidly and produce various types of soft rots with usually an abundant growth of aerial mycelium. The fungus mycelium also penetrates deeply into the decayed portion of the plant.

The following are some of the types of spoilage produced:

Type of Spoilage	Organism
Soft rot of tomatoes	*Rhizopus nigricans.*
Soft rot of strawberries	*Rhizopus nigricans.*
Black mold of bread	*Rhizopus nigricans.*
Soft rot of fruits	*Mucor racemosus.*

The asexual spores, sporangiospores, produced in sporangia on the aerial mycelium serve to spread the contamination. These spores are produced in countless numbers and remain viable over long periods of time. There are many forms of the phycomycetes which are of economic importance as plant pathogens. These include the downy mildews, the white rust of crucifers, and the late blight disease of potato, which cause considerable loss to food crops infected by these organisms.

4. Ascomycetes—Sac-fungi.

This class of fungi comprises almost half of the known forms of the fungi. Up to 1925 over 37,000 species had been described. The chief characteristic of these forms is production of a fruiting body called an ascocarp. In these ascocarps there may be one to several sac-like cells, called asci. Each ascus produces from four to eight spores called ascospores. These spores correspond to the sexual type since their formation depends on the union of nuclear material from special strands of the mycelium. There are two general types of ascocarps produced:

1. APOTHECIUM—more or less cup-shaped bodies in which the ascus-producing layer is exposed.
2. PERITHECIUM—a partially or entirely closed body in which the ascus layer lines the inner surface.

The asci produced in the ascocarps also vary somewhat in form. The ascocarps are used as a basis for classifying the fungi of this class. In addition to the ascospore formation all of the forms also produce asexual spores, conidia, on the vegetative mycelial growth. The following table lists some of the rots of various fruits caused by ascomycetes:

HOST	COMMON NAME	SCIENTIFIC NAME
Citrus fruits	Blue mold	*Penicillium italicum.*
		Penicillium digitatum.
Apple and Pear	Blue mold	*Penicillium expansium.*
Stone fruits	Brown rot	*Sclerotinia fructicola.*
Fig and Date	Smut	*Aspergillus niger.*
Orange	Sooty mold	*Meliola penzigi.*

BROWN ROT OF STONE FRUITS, such as cherries, prunes, plums, peaches, and apricots is a good example of a common form of rot caused by an ascomycete. The infection on the fruit is very characteristic. This infection is produced by the asexual spores. The spores produced on the decayed fruits are a means of rapid spread of the disease. The ascospore stage is produced in the spring after the fungus survives the winter on old fruit which are below the surface of the ground.

Fruits which are infected with brown rot decay rapidly and the mycelia penetrate deeply into the fruit tissues. Fruits affected with brown rot are unfit for food purposes.

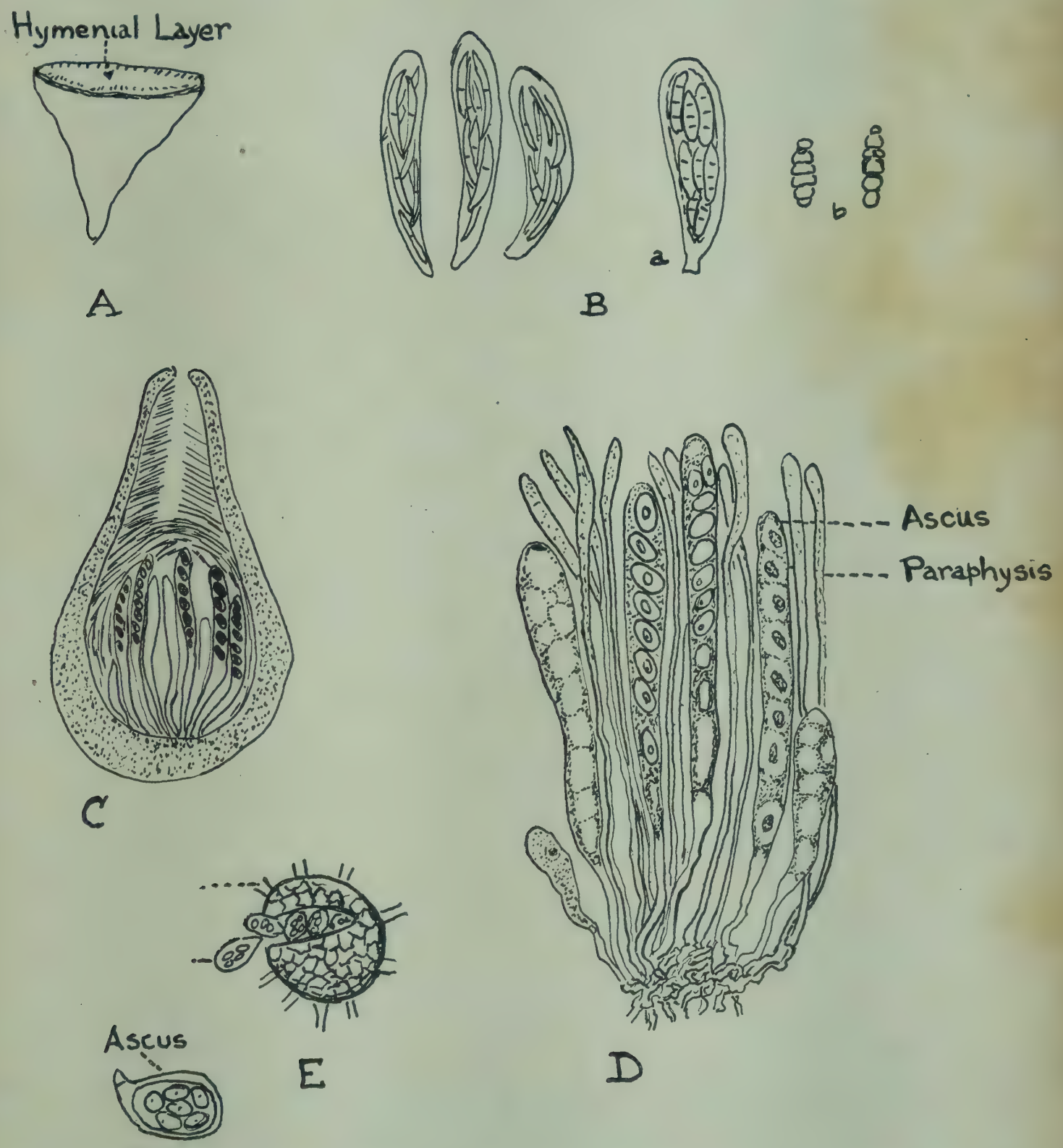

FIGURE 95.—Ascomycetes, types of ascocarps and asci. *A*, Apothecium; *B*, Asci with ascospores; *a*, ascus, *b*, ascospores; *C*, Perithecium; *D*, Portion of the hymenial layer of an apothecium showing asci and paraphyses; *E*, Perithicium of a powdery mildew. (From: *B, C, D*—Gauman-Dodge, Comparative Morphology of Fungi. By permission of McGraw-Hill Book Co.)

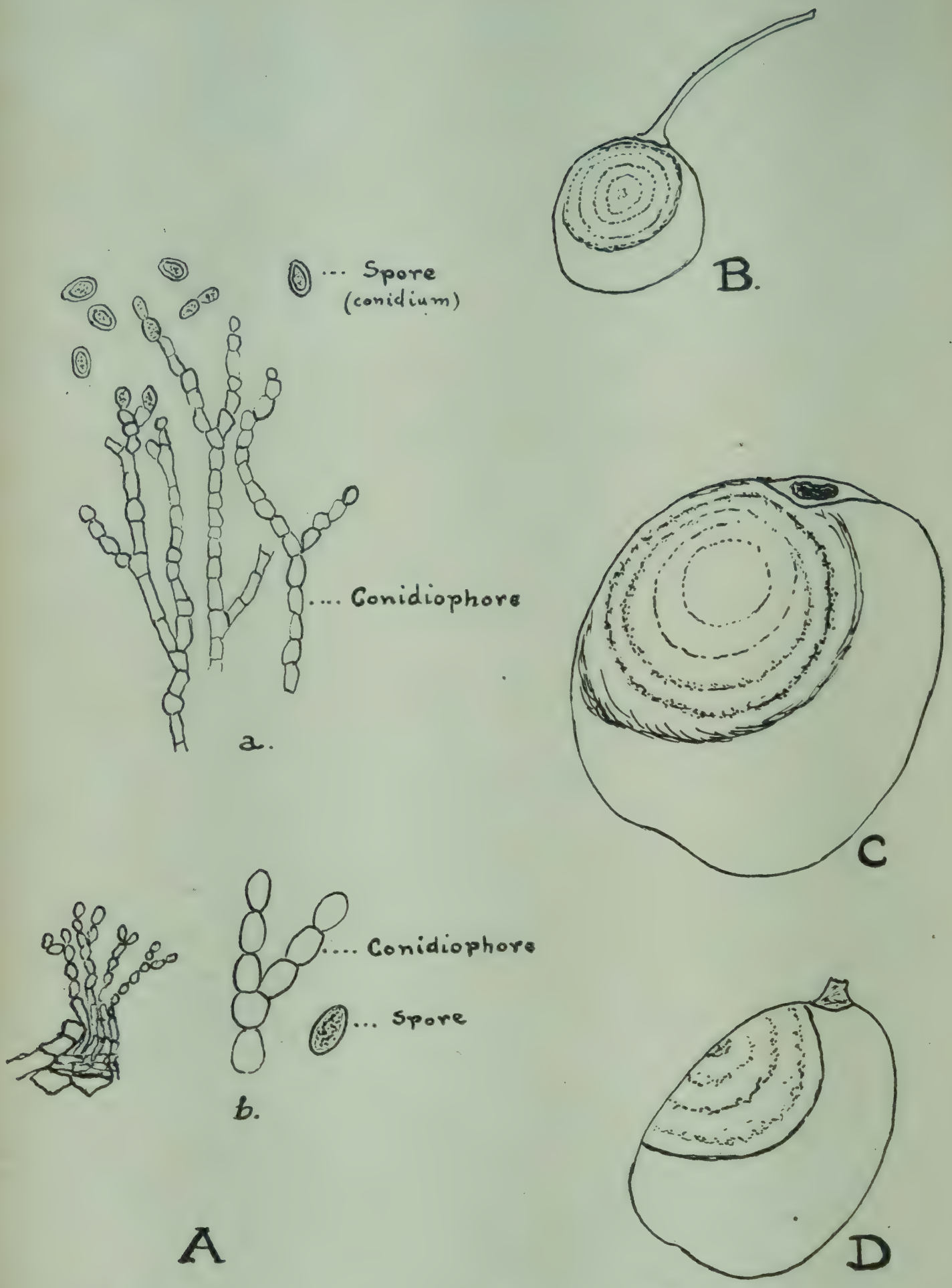

FIGURE 96.—Brown rot of stone fruits, *Sclerotinia fructicola* (Wint.) Rehm. *A*, Spores and conidiophores. *a, b; B*, Cherry; *C*, Peach; *D*, Plum. (From: *A. a*—Owens, Principles of Plant Pathology. By permission of John Wiley & Sons; *A. b*—Duggar, Fungous Diseases of Plants. By permission of Ginn & Co.)

The molds of citrus fruits caused by *Penicillium italicum* and *P. digitatum* are very common on fruits in storage. Under favorable conditions they develop rapidly and soon produce decay throughout the whole fruit. The asexual spores which are produced abundantly are the means of spreading infection among the fruits.

5. Basidiomycetes—Basidum Fungi.

These fungi include the forms known as mushrooms, or sometimes erroneously called toadstools, both terms referring to the same type of fungus. There are also two other groups of fungi in this class, (1)

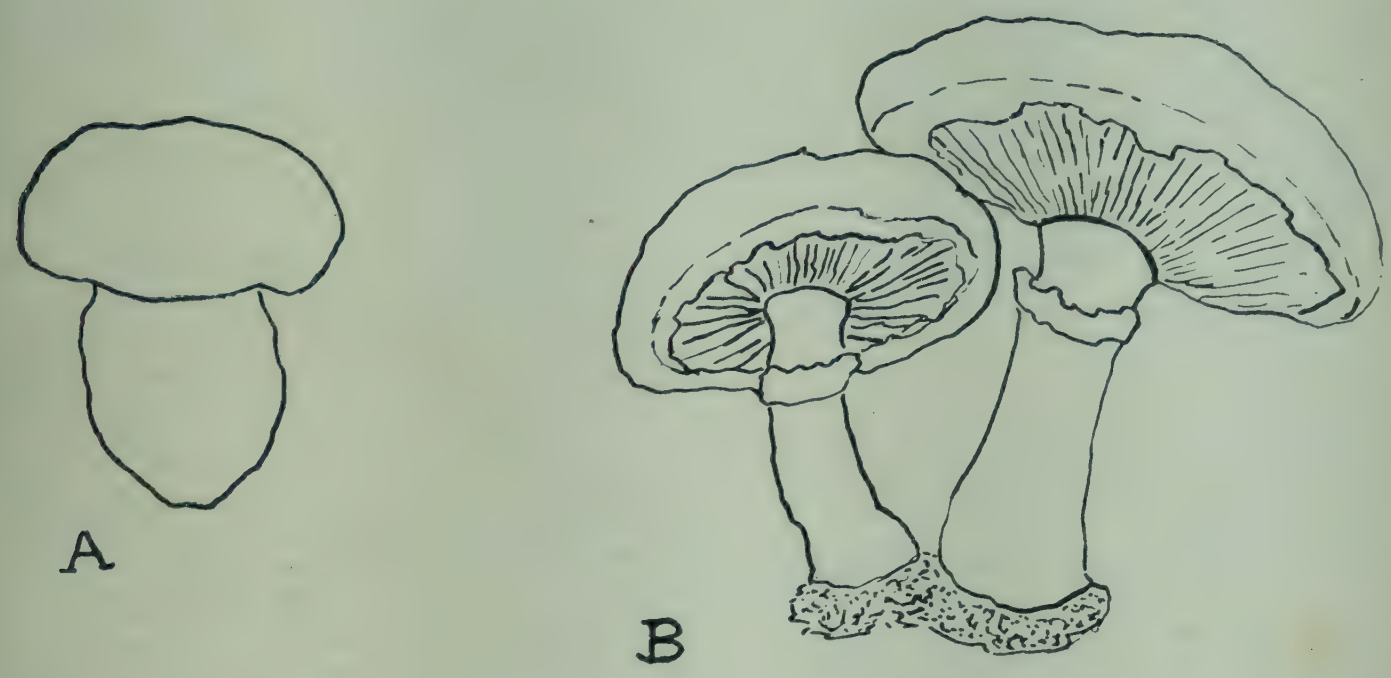

FIGURE 97.—Mushrooms. *A*, "Button" (immature) stage; *B*, Mature mushrooms; *C*, Portion of spore-bearing layer (gills); *D*, Spore and basidium; *E*, Section through mature mushroom. (*B* from Pennsylvania Agric. Expt. Sta. Bull. 392.)

Rusts and, (2) Smuts. All of these forms produce a special cell structure called a BASIDIUM, on which the BASIDIOSPORES, are produced. The structure of the basidium varies in the different groups in this class. There are usually four basidiospores produced on each basidium. The group which includes the mushrooms and puff balls produces a complex fruiting body called a SPOROPHORE. This sporophore constitutes the mushroom or puff ball as it is commonly seen in woods and fields. There are various forms of these sporophores.

The common cultivated mushroom *Psalliota campestris,* and various species of wild types of mushrooms are used as a source of food. Some forms of puff balls are edible in the young stages. CALVATIA, the giant puff ball, is delicious eaten young, when the interior is perfectly white. The commercial production of mushrooms is a well-developed enterprise. These mushrooms are sold as fresh or dried material and as canned stock. Many forms of edible wild types are used as dried mushrooms. Dried mushrooms were imported into this country up to the time of the war from China, Japan, Italy, France, and other countries. At the present time, domestic types of wild forms are being used for this type of product.

6. Fungi Imperfecti—Imperfects.

These forms are grouped in this class because of the fact that the asexual or vegetative spore formation is the only known type of reproduction. No sexual organs or method of nuclear fusion, resulting in the formation of sexual spore, are known to exist in the life cycle. Many of these forms are the cause of decay or rot in various fruits and vegetables. All forms possess a well-developed septate mycelium and produce abundant spores. These spores may be produced scattered over the mycelium or in special fruiting bodies called pycnidia. There are listed below some of the rot-producing fungi of the Imperfects:

HOST	COMMON NAME	SCIENTIFIC NAME
Apple	Blotch	*Phyllosticta solitaria.*
Citrus	Scab	*Gleosporium fawcettii.*
Citrus	Stem-end rot	*Phomopsis citri.*
Tomato	Fruit rot	*Phoma destructiva.*
Tomato	Nail-head spot	*Alternaria tomato.*
Tomato	Anthracnose	*Colletotrichum phomoides.*
Beans	Anthracnose	*Colletotrichum lindemuthianum.*
Carrots and other vegetables	Soft rot	*Sclerotium rolfsii.*
Peach	Scab	*Cladosporium carpophilum.*
Strawberries and other fruits	Gray mold	*Botrytis* sp.
Strawberries	Hard rot	*Rhizoctonia* sp.
Pear	Soft rot	*Penicillium* sp.
Tomato	Green mold	*Cladosporium* sp.
Tomato	Soil rot	*Rhizoctonia solani.*
Tomato	Late blight rot	*Alternaria solani.*
Tomato	Ring rot	*Melanconium* sp.

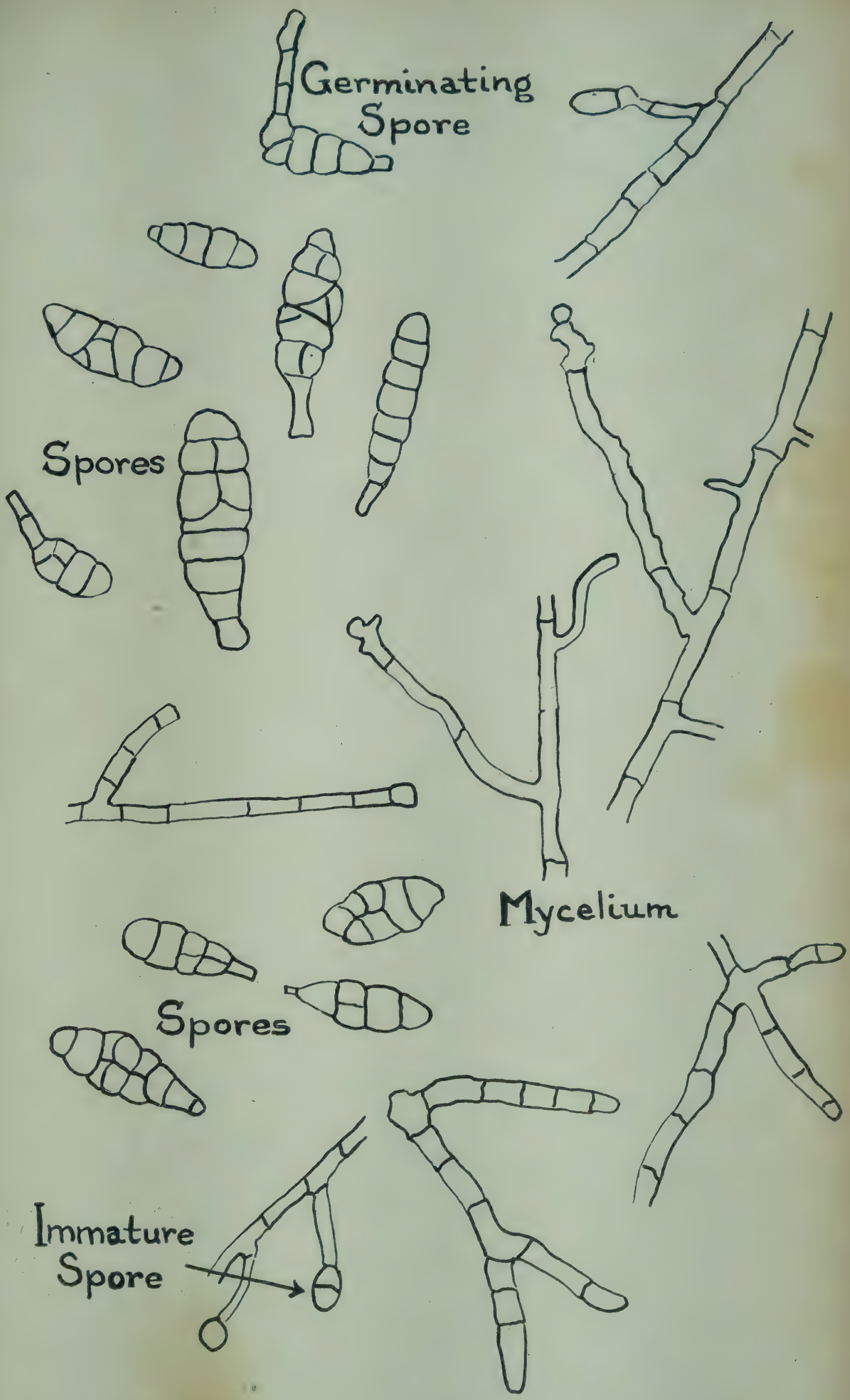

FIGURE 98.—*Alternaria solani.* Mold mycelia and spores from decayed area on a tomato.

7. Physiological Diseases

Plants will sometimes show symptoms of a diseased or abnormal condition which cannot be attributed to any parasitic organism. Studies of these abnormalities have shown them to be due to certain disturbances in the normal physiology of the plant caused by non-parasitic agents. Unfavorable weather conditions, certain mineral deficiencies, and improper storage conditions are some factors which may so upset the normal physiological processes of growth and maturation as to cause characteristic lesions and symptoms of decomposition. Where fruits or vegetables are thus affected they are more subject to attack by pathogenic organisms. Rot or decay is then caused by the organism which gained entrance to the host through the physiological injury.

The following lists some of the types of diseases of this group:

HOST	NAME OF DISEASE	CAUSE
Tomato	Blossom-end rot	Drought conditions.
Tomato	Growth cracks	Abundant moisture and high temperature.
Tomato	Sunscald	Hot, dry weather.
Tomato	Sulfur dioxide injury	Sprays.
Beets	Black spot	Boron deficiency.
Apples	Water-core	Excessive moisture and heat.
Apples	Bitter pit	Climatic and soil conditions.
Apples	Scald	Conditions in storage.
Potatoes	Black heart	Improper air conditions.

C. Characteristics of Some Common Fungi Causing Decomposition

There are some forms of fungi which are commonly encountered in various foods and food products in a fruiting or spore-bearing condition. For example, boxes of fresh strawberries are sometimes noted in which the berries contain a webby, cottony growth. Microscopical examination would show the type of fungus involved. Many of these fungi are closely related types and can be distinguished from each other only by the nature of their spore production. This may be illustrated by the following examples:

1. The Common Blue and Green Molds Produced by Penicillium and Aspergillus.

Both these forms have a similar type of mycelial growth but they can be readily distinguished from each other by the structure of the spore-bearing organs, the conidiophores.

2. Mucor and Rhizopus Molds.

Both forms produce a coarse non-septate type of mycelium but differ in the kind of sporangia (spore-producing bodies) produced. The sporangia in both cases are produced on stalks called sporangiophores. In the case of *Rhizopus*, the sporangiophores arise from a single point forming a group of stalks which are connected with another group by

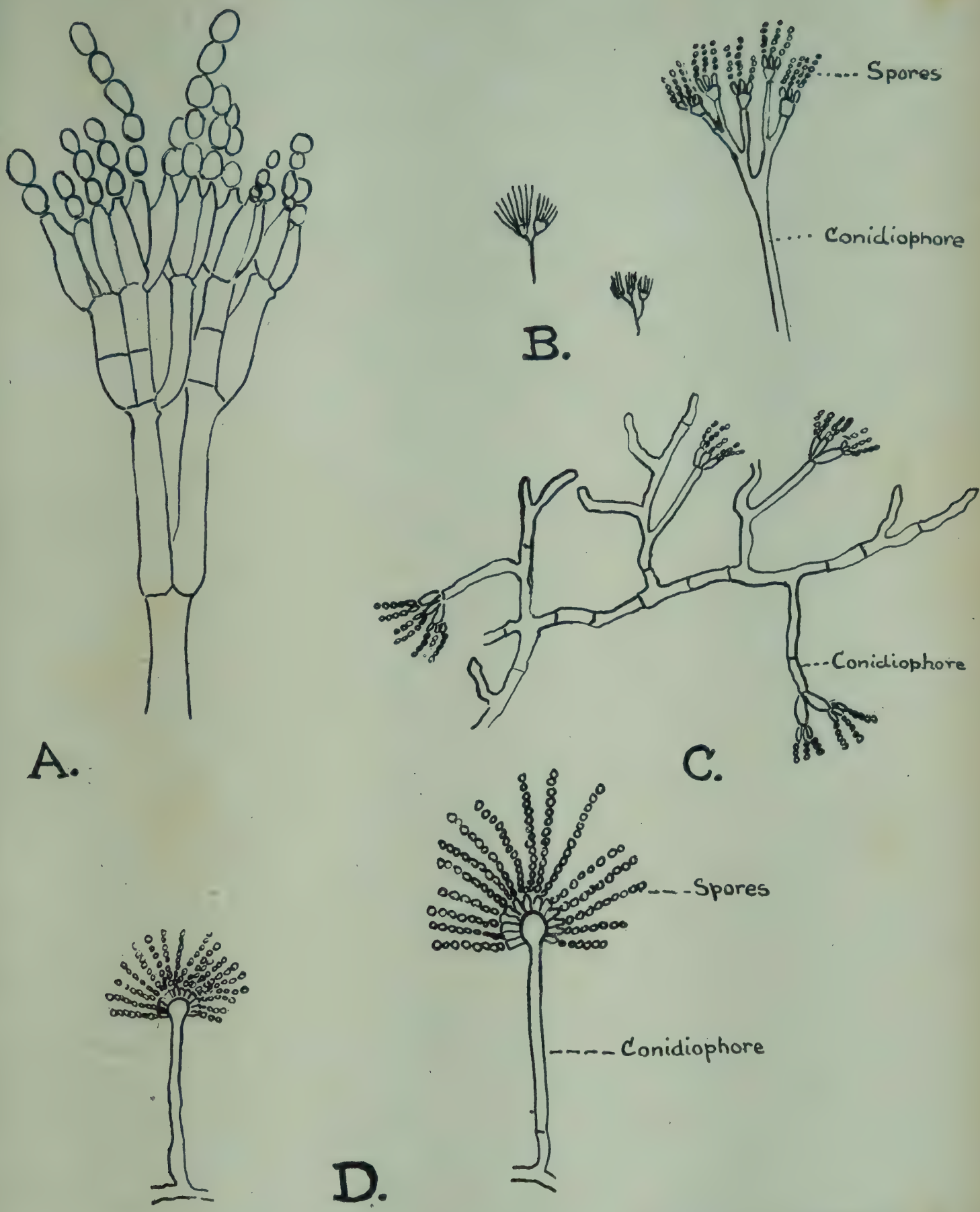

FIGURE 99.—Common blue and green molds. *A, Penicillium commune; B, Penicillium* Sp.; *C,* Mycelium; *D, Aspergillus* Sp. (From: *A*—B. A. I. Bull. 118, U. S. Dept of Agric.; *C*—Holman and Robbins, Textbook of General Botany. By permission of John Wiley & Sons; *D*—Stevens, Fungi Which Cause Plant Disease. By permission of The Macmillan Co.)

a stoloniferous hypha. In the case of *Mucor,* sporangiophores may arise as branches from any part of the mycelium and there are no stoloniferous hyphae.

3. Macrosporium and Alternaria.

It is sometimes possible to find these organisms in a fruiting condition, whereby they may be distinguished. The spores of both genera

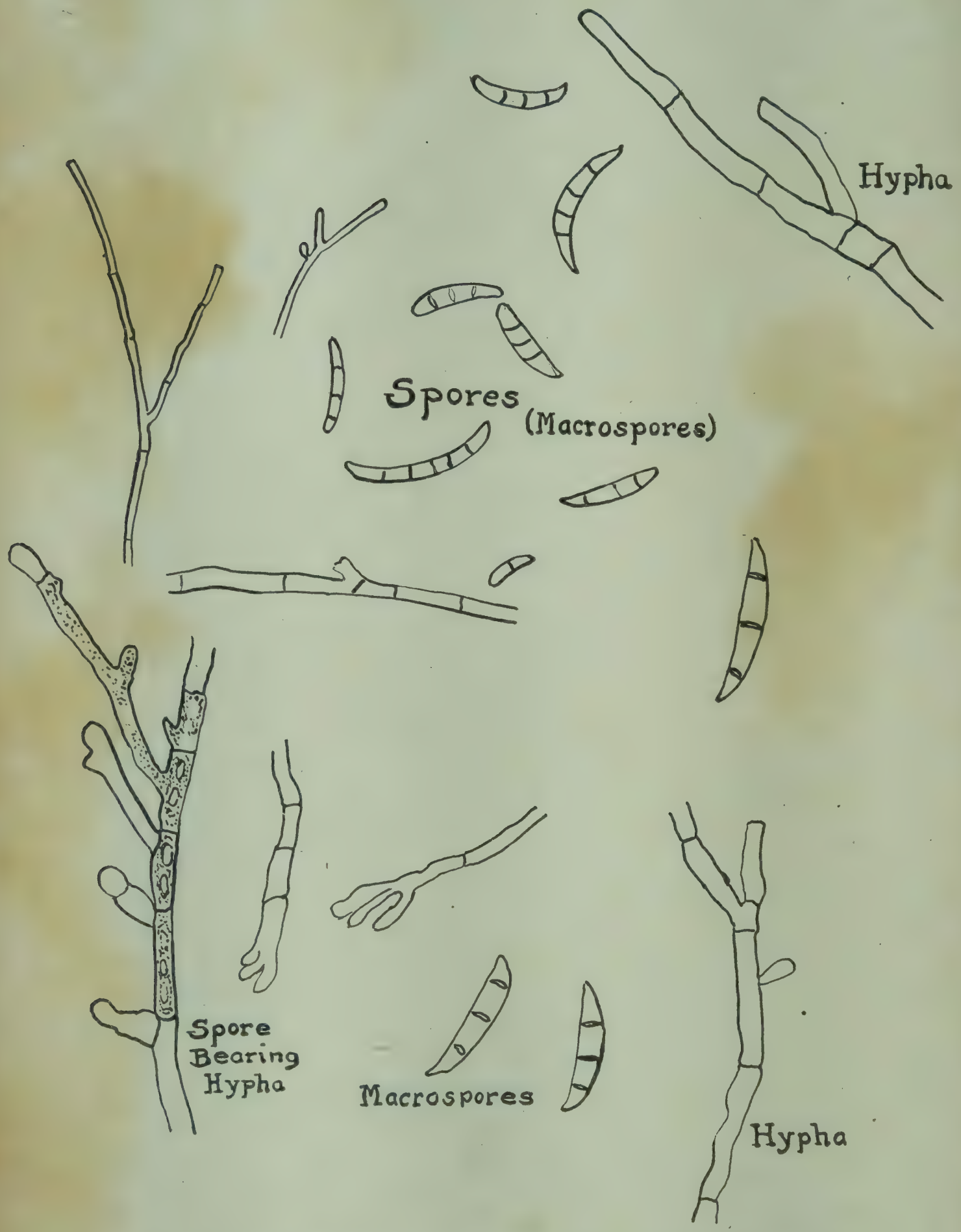

FIGURE 100.—Fungi imperfecti—*Fusaria* sp. from tomatoes.

are similar in appearance and unless the mode of their formation is noted it is impossible to tell them apart. The spores are Indian-club-shaped with crosswalls and usually of brown to olivaceous color. In *Macrosporium* the spores are produced singly on the mycelium, while in *Alternaria* they are produced in chains.

4. Other Molds.

Some other molds may be readily recognized by the characteristic structure of the spores or type of spore formation. These include organisms as *Fusarium, Botrytis, Oospora* (oidium) and *Cephalothecium.*

Fusarium. The many species and varieties of this genus produce various types and colors of mycelial growth but the general type of spore is characteristic of the group. There is some variation in the spore size and septation but all have a sickle or lunar-shaped spore.

Botrytis. This fungus which causes gray mold of various fruits and vegetables is readily recognized by the characteristic type of spore production. The spores are produced in grape-like clusters attached to the ends of short branches of the conidiophores.

FIGURE 101.—Comparison of three common molds. *A, Botrytis; B, Oospora; C, Cephalothecium.*

Oospora (oidium). Spores of this mold are produced as segments of the mycelium and become detached cells of various sizes of any hyphae of the mycelial growth. Figure 101 *B* is a drawing of *Oospora* mold from dirty factory machinery.

Cephalothecium. This organism produces a pink rot due to the clusters of rose or pink-colored conidiophores and conidia arising from the mycelium. The conidia, which are 2-celled, form in clusters at the end of the conidiophore.

YEASTS. These organisms belong to the class ASCOMYCETES. They are often found in decaying fruits and vegetables growing in rots produced by bacteria or other organisms. They are single-celled and reproduce by a vegetative process called budding. The buds often produce long chains of spores. Ascospores have been found in some forms. The yeasts belong to the genus *Saccharomyces.*

FIGURE 102.—Yeast colonies on dates.

D. FOOD PRODUCTS AFFECTED BY MOLDS

Molds of many kinds cause decomposition in vegetables and other organic material. In many cases the organisms penetrate the host tissues and in practically all cases so alter their structure that their orignal character is lost. In some instances the symptoms resulting from infection by molds may not be marked in the early stages of growth while in others the effect is almost immediate as, for example, the rotting of fruits by *Rhizopus*.

1. Citrus Fruits.

BLUE AND GREEN MOLDS. The most prevalent form of decay of citrus fruits is caused by the green and blue molds.

The first sign of either mold is the appearance of a water-soaked, soft area. This spot enlarges and soon a white growth of mold appears. This is followed by a development of an olive-green or blue-green powdery mass of spores. Usually both molds are found together on the same fruit but it is more common to find the green mold developing after the blue one has initiated the breakdown of the tissues. The blue mold is usually within the fruit tissues and penetrates deeper than the green mold, even producing spore masses in the inner tissues as well as on the surface.

These rots are caused by the fungi *Penicillium italicum* (Wehmer), Blue mold, and *Penicillium digitatum* (Sacc), Green mold. Reproduction and spread of these molds is by means of asexual spores. Since no sexual spores have been found in the life cycle of these forms, they are classified as FUNGI IMPERFECTI.

Aspergillus Rot. A minor type of rot closely related to the blue and green mold is a form of soft decay caused by *Aspergillus niger* (Van Teigh).

The decayed area is similar in many respects to the type caused by the blue and green molds. However, the rot does not develop rapidly except where the fruit is held at relatively high temperatures.

Spores are produced on the decayed area in powdery, black-brown masses.

The fungus *Aspergillus niger* completes its life cycle by means of asexual spores, conidia, and is classified in the FUNGI IMPERFECTI.

Stem-end Rot. Stem-end rot, as the name implies, is a pliable, leathery type of rot at the stem-end of the fruit. The first sign of infection is a slight softening of the rind. This form of decay may be caused by either of two fungi: (1) *Diplodia natalensis* or (2) *Phomopsis citri*. These two forms produce a similar type of rot in the fruit. Often the same fruit contains both fungi.

The phomopsis rot starts with a slight softening of the stem end with no discoloration. As the decay develops, an "off-color" shows on the fruit which becomes brown or drab. By the time the decayed portion involves about one-third of the fruit the color becomes darker but the rind is still pliable and not easily punctured by the finger. The fungus develops rapidly along the center where the segments of the fruit join and also along the inner white portion of the rind. The fungus penetrates slightly into the juice sacs but not as rapidly as in the albedo.

The rot caused by the *Diplodia* fungus produces a similar type of rot to that caused by *Phomopsis*. The decayed area darkens much faster and increases more rapidly. When about one-third of the surface is involved, decay streaks advance downward ahead of the main decay. When most of the fruit is infected the color is a dark olive green to blackish-brown. Both of these forms develop on the dead twigs and branches of the tree where the spores are produced in abundance and which are the source of infection of the fruit. The spores are spread from the infected areas during the warm rainy weather.

Infection of the fruit takes place while it is still on the trees but it usually develops after the fruit has dropped or been picked. Its growth in the fruit depends on weather conditions and length of time the fruit is held. They may cause decay of the fruit during transit or after the fruit reaches the market. Both of these forms belong to the class Fungi Imperfecti, since their spore form represents the asexual or vegetative stage. Stem-end rot may be encountered in fruits used in the manufacture of juice and thus become a source of mold contamination in the finished product.

Oospora Rot. (*Oospora* sp.) This type of rot is usually found associated with soured, yeasty fruit. Cracked or broken fruits in storage bins soon become infected with *Oospora* and yeast organisms. These organisms break down the tissues and expose it to attack by secondary forms. Oospora rot, also called sour rot, produces a soft, slimy, watery type of rot and in moist, humid atmosphere develops quickly and reduces the fruit to a putrid, sour, and dirty mass. This condition of the fruit is especially attractive to fruit flies and forms a breeding place;

the decayed areas are often filled with the eggs and maggots of these flies. The fungus produces an aerial mycelium which develops chains of spores. These spores are oval to oblong and have obtuse or nearly square ends and when growing on fruits have oil globules and granular contents. The mycelium may also break up into many spores, segments of the hyphae becoming spores. This fungus is closely related to the mold found in moldy cream, *O. lactis,* as it has similar morphological characters.

Sour rot is most frequently found in fruit held in storage for a considerable time and in fruits invaded by other organisms. Mechanical injuries or other skin damages are usually necessary for invasion by this form. The rot spreads rapidly by contact of fruits and also by fruit flies that may carry spores or bits of mycelium among the fruits. The use of such fruit for juice manufacture is a potent source of contamination. The *Oospora* mold also readily grows in and around factory equipment, especially in the pipelines carrying the juice, and on the juice-extracting machinery. Unless the factory equipment is frequently cleaned, this mold may develop rapidly and considerable quantities be found in the finished product. It is readily recognized in the juice by the feathery-like groups of branched hyphae.

2. Dairy Products.

Molds are found in dairy products as undesirable contaminants. These present a sharp contrast to the flavor-producing molds which are introduced to ripen and impart particular flavors to certain types of cheese.

Molds and Cheese. The Camembert and Roquefort types of cheese are examples of cheeses which require the action of certain specific molds in their manufacture. Camembert cheese is produced through the activity of *Penicillium camembertii,* while the growth of *Penicillium roquefortii* is required to produce the flavors characteristic of Roquefort cheese and the other blue types of cheese. In both cases it is essential that the curd be made ready for the action of the mold in definite, carefully-controlled steps varying in detail according to the type of cheese to be produced. After the curd has been prepared, it is inoculated with spores of the particular mold needed. The procedures involved in the production of the mold inoculum are also very carefully controlled in order to keep out contaminating organisms. Lack of care at this point would result in spoilage of the cheese later on. The growth of the molds in question on the carefully prepared curds is controlled further by exposure of cheeses to definite and standardized temperatures and humidities. When the requisite factors have been secured the cheese ripens into the characteristic end product. The type of mold, the type of curd and its preparation, and the conditions of ripening must all be right for the desired product to result.

On the other hand, it is possible for any type of cheese to be spoiled and made unfit for food by contamination with molds. For example, the occurrence of the common *Penicillia* molds growing throughout pockets and crevices in Cheddar cheese would without question spoil the cheese.

Molds in Cream and Butter. In this discussion we are interested in butter made from sour cream by commercial processes. The cream for

a churn of butter usually will have originated from many different and widely separated farms, the amount of cream coming from any one farm varying usually from 1 to 10 gallons. On the farm the cream may be accumulated twice daily by separation from fresh milk either by means of a centrifugal separator or by the gravity process. The fresh cream is added to the storage lots either after an initial period or directly from the separating process. The cream is transported to the creamery after a storage period on the farm varying from a few days to 2 weeks, or even longer.

Molds enter the picture first as contaminants in the milk. At this point, air-borne molds may infect the milk from dust in the barn or from the body of the cow or from the utensils. The most abundant molds encountered in freshly-drawn milk are the typical air-borne types such as *Mucor* sp., and *Aspergillus* sp.

Contamination of Cream with Oospora. *Oospora* mold is the most common mold in stored cream, but it has not been found floating in the air or in freshly-drawn milk where the utensils have been properly cleaned. *Oospora* has been found in some samples of cow manure and might conceivably get into milk from this source, or since it has been found in soil it might enter milk from that source. The most abundant source of *Oospora* mold, however, and the one most likely to act as a true contaminant, is the batch of cream accumulated during the previous storage period. Layers of *Oospora* mold contain millions of spores and any utensil used for stirring this cream will contaminate fresh cream if the utensil is not thoroughly cleansed and sterilized. Once mold has been allowed to start, each storage lot of cream presents a cycle of increasing concentration of mold spores. To eliminate mold growth in future lots it is therefore necessary to clean up and scald all equipment used for the previous lots of cream. To stir a badly molded lot of cream with a dipper and then merely to rinse this dipper in a common receptacle along with the separator parts or buckets or other utensils used for the new batch of cream is simply to insure a thorough seeding of the fresh cream. A wash rag used in this half-hearted rinsing operation may contaminate every utensil so that mold growth is sure to result. Once mold does become established it is necessary to make a sharp break and a thorough clean-up between the last batch of cream and the next.

A heavy contamination with mold and a high summer temperature will result in the formation of a layer of mold in less than 24 hours. If stirred in, new layers will form, or if left undisturbed, the mold will continue to grow, eventually forming a heavy wrinkled mat of mold. On the other hand, many farmers regularly market cream, even at weekly intervals, which shows little or no mold. Experimentally it has been found that by use of clean utensils the initial *Oospora* inoculation is held to a very low level and by fostering the normal souring of the cream with a temperature of around 20° C. mold layers do not develop within a period of 1 week.

Since insanitary conditions lead also to the decomposition of cream, it is not surprising that mold and decomposition are associated in the great majority of cases. In some instances poor cans of cream show no mold while some moldy cream fails to show decomposition characteristics. It should be borne in mind in the latter instance that mold layers

may produce a putrifying condition on the surface but that this condition may be masked when the cream is stirred. Because of the association of mold with decomposition, the percentage of decomposed cream in the churn will be reflected by the relative density of the mold in the butter.

Making the Mold Count. There are a number of points which the analyst should particularly bear in mind in making the mold count by the M4E(1) method. It will be noted that this method as it appears in the Manual provides for weighing the gum solution as well as the butter. The question of the viscosity of the gum solution used for diluting the butter is important. At the present time the method calls for the use of a 0.75 percent solution of carob bean gum or a 3 percent solution of pectin with the provision that other gums may be used providing a final solution is obtained which has a viscosity approximating that obtained with the specified gums. It was necessary to provide for the use of discretion on the part of the analyst in making a gum solution because of difficulties encountered in obtaining the gums which were specified in the Methods of Analysis of the A.O.A.C., 5th Ed. The point to bear in mind in making a selection of a gum solution is that with certain types of butter a heavy gum solution will give an emulsion containing very fine fat globules which will tend to hide the mold filaments. With certain butters, particularly those with a high mold count, it has been found that the heavier gum solutions produce the undesirable emulsion almost immediately after the mixing of the gum and solution has been started. It is specified in the method that the fat globules in the final mixture should be from 0.1–0.2 mm. in diameter. It will therefore be necessary in these particular butters when encountered to use a gum solution of such a viscosity that the fine fat globules are not produced.

For mold counting, the source of illumination should be adjusted according to the method described in the Manual. Care should be taken not to obscure the mold filaments with too much light. One of the difficulties encountered by new analysts is the use of an improperly adjusted microscope and source of light. The analyst should experiment with the light adjustment until he is certain that all mold filaments in the field are visible. The use of stain as provided for as an alternate procedure (M4E(1)(a)) may be of some assistance to the analyst in this regard.

The mold in commercial butter usually occurs as rather short filaments and quite frequently the mold is of a very fine nature. The reason for this appears to be that the protoplasmic material within the mold has disappeared to a large extent, leaving only the tubular structures. However, in practically every case each separate mold filament will show some protoplasmic material clumped together in some part of the filament. Cross walls usually occur within these separate filaments, at least in one or two places, and careful study will show that while the mold wall has shrunken down in most of the filament it will not be shrunken at the cross wall.

3. Dried Capsicum Peppers.

Dried sound fruit is shriveled and of a more or less uniform color, while the texture of the skin is such that it appears as if lacquered. The diseased fruits or culls are not uniform in color but present patches

of black, wine-red, bright red, greenish-yellow, or straw-yellow colors in varying proportions, depending on the extent and nature of the disease. The texture of the outer skin is usually dull and drab in appearance.

Many fungi have been isolated and described in the literature as causing fruit rots in Capsicum peppers. Some of the more prevalent diseases encountered are:

(a) Alternaria rot or black spot appears as large discolored lesions covered with a dark brown to black velvety mycelium producing numerous large, olivaceous, muriform spores.

(b) Botrytis rot appears in the interior of the pod as a number of rounded to irregularly shaped, brown, flat or slightly cup-shaped, crust-like sclerotia of varying size consisting of masses of brown conidia and mycelia. The seeds similarly show infection. The organism responsible, *Botrytis cinerea* Pers., is a destructive parasite of peppers as well as a serious storage rot organism.

(c) Anthracnose is responsible for some discoloration, evidenced by yellow to slate-colored sunken lesions, often covered by a black velvety growth of *Alternaria*. The acervuli and elliptical conidia of the causative organism, *Colletotrichum nigrum* Ell. and Halsted, are only occasionally seen.

(d) Internal rot is the name applied to various saprophytic molds growing in the interior of the fruit, which are scarcely visible on the exterior. Quite often, an apparently sound pepper, when opened, reveals a velvety mat of white to slate-colored mycelia firmly attached to the inner wall of the pericarp. The filaments are usually hyaline, scarcely septate and measure 2–4 μ in diameter. Other secondary fungi such as *Mucor* and *Rhizopus* may find their way inside the fruit through an inconspicuous lesion or through a dead pistil.

(e) Blossom-end rot, sun-scald, and frost-injury, although non-pathogenic injuries, open the way for secondary or saprophytic molds to attack the fruit. *Alternaria* sp., which is found quite commonly in much of the culled dried fruit, is itself a weak pathogen but becomes well established when coming into contact with mechanically injured tissue and quite often follows injuries caused by other organisms. Blossom-end rot caused by an irregular water supply is evidenced in the dried fruit by a discolored papery-white or straw-yellow area. This originally water-soaked area may dry out without becoming infected but in many fruits examined the spots were usually infected with various fungi.

The rots described above are easily detected in the whole pods and are obvious to the analyst as unfit material. Their detection, however, in finely ground capsicum products as chili powder, paprika, red pepper, etc., requires a different approach. Here the evidence of the rot is present as microscopic fragments of the mycelium distributed throughout the discolored area of infection. A mold-counting procedure (M 7 (a) (2)) has been devised which makes it possible to determine from a microscopic count of the mycelial fragments the relative amount of rotten material that has been included in a ground product. Although the technique involved in making the count differs in some details from those made on other products the procedure and principles are essentially the same and are more fully discussed in the section dealing with tomato products.

4. Berry Fruits.

Molds Causing Rots in Strawberries. Strawberry plants and fruits are subject to infection by fungi. The fungi attacking the fruits produce various types of rots. These rots cause considerable damage to the fruit crop and may be a source of rot contamination in food manufacture unless careful sorting is practiced at the factory to remove infected berries. Fruit rots cause greater loss to growers than all other diseases combined. Widespread damage is usually caused following periods of prolonged rain and cloudy weather, with favorable temperatures suitable for rapid growth by the particular fungus. These rots show certain characteristics by which they may be easily recognized.

(a) Gray Mold—*Botrytis*. This fungus is probably the most common and widely spread rot of strawberries. It affects both the green and ripe fruit. It first shows up as a light-brown soft spot. The fungus develops and grows throughout the whole berry and causes the berry to dry up and become tough and leathery. Following this there soon appears a grayish, or dusty-like mycelial growth from which the mold is named. Infected berries, if not carefully removed before shipping, will develop the mold during transit and spread the infection to surrounding fruit. The fungus often affects both blossoms and leaves and thus damages the entire plant. Microscopic examination of the mycelial growth shows the characteristic type of spore production by this fungus.

(b) Hard Rot—*Rhizoctonia* sp. This fungus produces a hard, brown-colored decayed spot on the berry on the side next to the soil. The uninfected portion is not changed in color or taste by the fungus growth. Since the organism lives in the soil, the rot on the fruit occurs at the spot where the berry comes in contact with the soil. The growth of the fungus in the fruit is rather slow and its spread from diseased to healthy fruit under good refrigeration is doubtful. Some fruits infected in the field may not show a decayed area until after the fruit reaches the market.

(c) Tan Rot—*Pezizella lythri*. The fungus causing this type of rot produces a sunken, soft, tan-colored spot on both green and ripe fruits. The fungus growth penetrates deeply into the fruit tissue, forming a core of mycelia that can be removed from the decayed area intact. The size of the spots varies from ¼ to ½ inch on the green fruits, and on the ripe fruits is much larger as a result of the rapid growth and spread of the fungus. Infection of the fruit takes place only through mechanical injury.

(d) Leather Rot—*Phytophthora cactorum*. This rot starts as a field infection and may be found on fruit on the market. The decayed portion has a slightly bitter taste. The decayed area soon becomes tough and leathery. Berries in all stages of development may become infected by the fungus. The growth of the fungus produces various types of characteristic symptoms. Ripe berries have hardly any change of color over the decayed portion while less mature fruit has a range of color from yellow, dark brown, or purple. The affected areas are not disintegrated and are usually soft at first, becoming tough and leathery. A cross-section of an infected fruit shows a brown discoloration of the water-conducting systems. The fungus is capable of causing infection

through uninjured skin. The disease is usually prevalent during wet, humid weather and the disease level is sometimes called water-soak disease.

(e) Leak—*Rhizopus nigricans*. The fungus is widely spread, occurring as a rot-producing organism on many fruits and vegetables. It is perhaps one of the most common forms of fungi causing decay in stored fruits and vegetables. The name "leak" is associated with this rot because as the fungus spreads through the fruit it breaks down the tissues and allows the juice to escape. Soon after infection takes place there appears a luxuriant growth of coarse white mycelium. The mycelium soon becomes dotted with white glistening heads (sporangia) which are the spore-bearing bodies. In the later stages of growth these become black. When mature, the sporangia liberate countless numbers of spores which are the source of infection of other fruits.

Most of the damage to the fruit takes place in transit or in storage. Berries showing no indication whatever of damage by this rot may be badly molded by the time they reach market. This is due to the presence of spores and favorable conditions for their germination and growth. The development and spread of the fungus is greatly favored by skin injuries to the fruit caused by improper handling or loading for shipment. Moisture and high temperatures also favor the rate of growth of the organism. Under refrigeration there is only a scant growth of the fungus.

Examination of Frozen Strawberries. The field rots are readily detected in the frozen produce since they are accompanied by marked discoloration of the berry. The essential thing to bear in mind is that if the fruit is allowed to thaw out while exposed to air, the rot in the berries may be masked by the moisture present, and a general darkening of the berries. It is necessary to overcome this effect before a satisfactory examination can be made. (See Method M8C.) To this end it is advisable to keep the fruit frozen until shortly before the time of examination and then to allow it to thaw. As soon as the fruit is sufficiently thawed it is placed under water and the water decanted and fresh water added to enable the worker to observe the berry readily. The berries should be completely covered with water.

Under the above conditions the rotten areas can usually be detected by their discoloration. While this is true for field rots, it is more difficult to detect fruit which has spoiled by action of *Rhizopus* mold. Here the berry may not show obvious discolorations and the analyst may overlook the presence of the spoiled fruit. Clues which should cause the analyst to make a further search for the presence of rhizopus-spoiled fruit are frayed and mashed berries and berries which hang together. The analyst should place a few of the suspected berries at a time under water in a small receptacle and examine them with a low power of the Greenough microscope. Strands and fruiting bodies of the *Rhizopus* mold can be detected in this way if present in significant amounts.

Molds Producing Rots in Berry Fruits Such as Raspberries. Blackberries, and Dewberries. Raspberries, blackberries and related plants are subject to attack by various fungi which may cause fruit rots. In general, all of these fruits are subject to attack by the same molds. These molds are common in many fruits and have been discussed in other sections. They include *Botrytis* sp., *Rhizopus* sp., *Penicillium* sp., *Phyllostictinia carpogena*, *Elsinoe veneta*, and *Cladosporium* sp.

Two types of rot not discussed elsewhere are the following:

(a) Anthracnose—*Elsinoe veneta*. This fungus attacks all of the raspberry types of plants producing the same general type of decay. It attacks the canes and fruits of the plant and in some varieties affects the leaves. The damage to the fruit comes from the infection of the drupelets; on young, green fruits the infected portion shows as tiny spots at the tip of each drupelet. As the fungus grows it increases the size of these spots and prevents the normal ripening of the fruit. The infected fruit remains small and becomes dried up and worthless. The fungus is classified as an ascomycete since the perfect stage, the ascospore, is known to exist in the life cycle of this form.

(b) Black Rot—*Phyllostictina carpogena*. A similar type of fruit rot is produced by this fungus on all forms of the raspberry group of plants. It produces a rot of the fruit that develops during transit or under storage conditions. The fungus attacks only over-ripe fruit penetrating into the flesh of the berry, and causes the berry to become soft and mushy. The fungus belongs in the class Fungi Imperfecti, since only the asexual conidial stage is known. The conidial spores are the means of spreading the disease.

The Examination of Raspberries and Similar Berries. (See method M8B.) Fresh berries which have molded can be readily noted on direct examination if the mold has reached the aerial stage of development, although the spoilage may be overlooked at earlier stages. The growth of *Botrytis* sp. on raspberries is a good example of this effect. The mold tends to grow into the berry tissue and cause a spoilage of the fruit. The condition at this stage can be detected upon close examination because of its light tan color. However, since the berry itself is darker than the discoloration this presporing stage may be overlooked. At the next stage of the mold growth typical aerial conidiophores are sent up through the berry surface and on these are born innumerable grayish spores. At this stage the moldy condition is obvious. In order to evaluate the condition of the fruit at the time of packing from the subsequent examination of the product, it is necessary to search for any aerial mold that might be present. When aerial mold on a berry is moistened, either by washing or mashing of the fruit, it seems to disappear. However, this effect is apparent rather than real as the mold does not wash off but simply becomes more or less transparent due to the effect of the wetting.

The field investigator should be particularly on his guard against drawing false conclusions from the examination of mashed or wet berries. Here, as in the case of tomatoes, he should examine the berries in the field containers and then transfer his attention to the sorting belt and finally to the sorted berries. Masses of berries held together by mold growth usually show some aerial mold and since all berries in such clumps contain mold the whole clump should be discarded.

In the laboratory the detection of the areial type of mold often requires care and patience. An obviously bad condition of the fruit in the plant rarely carries over into the finished product the degree of repulsiveness noted in the fresh fruit. To detect the aerial mold the berries are placed under water and a strong beam of light directed against the edge of the berry. The berry is examined with the aid of a hand lens and turned in such a way as to bring all surfaces of the berry successively into relief in the beam of light.

5. Diseases of Stone Fruits—Peaches, Cherries, Plums, Apricots.

Stone fruits are subject to attack by molds which cause varying amounts of damage. Some of the rots produced penetrate deeply into the flesh of the fruit, while other forms produce only skin damage with little or no penetration into the flesh. Some of the molds are commonly found on all the types of stone fruits.

The following lists some of the various fungi causing rots in stone fruits:

COMMON NAME OF DISEASE	CAUSAL ORGANISM
Green Mold rot	*Alternaria* sp.
Black Mold rot	*Aspergillus* sp.
Blue Mold rot	*Penicillium* sp.
Brown rot	*Sclerotinia fructicola.*
California blight	*Coryneum beyerionckii.*
Scab	*Cladosporium carpophilum.*
Gray mold	*Botrytis* sp.
Rhizopus rot	*Rhizopus nigricans.*

Brown Rot. The most important rot causing damage to the stone fruits is the brown rot caused by *Sclerotinia fructicola.* The fungus produces serious damage to the fruit, causing a decay which spreads rapidly through the fruit. The brown-colored rot is the characteristic feature of the decay. In the decayed areas an abundant growth of brown-colored, somewhat barrel-shaped spores occurs which spreads infection to other fruits. The growth and spread of the organism in the orchard depend largely upon weather conditions, the most favorable to its spread being warm, wet, or cloudy weather.

Though brown rot may originate in the orchard, it may also cause damage to fruits in transit or after they reach the market. Any damage to fruit causing breaks or cracks in the skin provides a means of entrance for the fungus. The disease on the fruit appears at first as a small light brown spot. This rapidly increases in size and under favorable conditions may bring about complete decay in 24 hours. As the decayed area increases the spot becomes dark brown in color and there appear in ring-like formation masses of grayish spores which serve to spread the infection to other fruits. The flesh of infected areas remains firm and the skin firmly attached.

The organism causing brown rot is a fungus belonging to the class *Ascomycetes.* The ascospores are produced in the spring on the ascocarps growing on dried and completely shriveled fruit. The ascospores carry infection to the fruit trees in the early spring.

Gray Mold—*Botrytis* sp. The fungus Botrytis which produces the gray mold rot is commonly found on all stone fruits. The organism usually gains entrance to the fruit through breaks in the skin though occasionally it has been found to penetrate sound epidermis. It produces an aerial growth of grayish-brown mycelium covered with clusters of grayish-brown spores. If the fruit is kept under moist conditions the mycelial growth is sometimes white and spreads rapidly through a box of fruit.

The fungus belongs to the class of fungi called the Fungi Imperfecti.

Blue Mold—*Penicillium* sp. The fungus producing this rot occurs on all types of stone fruits and is most destructive on fruits in transit

or on the market; it is seldom found attacking fruits on the tree. The organism is very common on all kinds of dead or decaying material and produces countless numbers of spores which are the source of infection of other fruits. Cracks or bruises in fruits serve as points of infection by this organism. Careful handling of fruit during packing, and shipping will reduce the loss of fruit decayed by this fungus.

Rhizopus Rot—*Rhizopus nigricans*. The fungus *Rhizopus nigricans*, producing this rot attacks chiefly fruits in transit and on the market. It produces much the same type of symptoms in all the stone fruits and under proper moisture and temperature conditions develops rapidly, producing a surface growth of white mycelium with gray or black-colored spore-bearing bodies, the sporangia. The fungus gains entrance only through breaks or bruises in the skin of the fruits and grows best at high temperatures. Careful handling of the fruits for shipping and proper refrigeration conditions tend to decrease the losses caused by this fungus.

6. Tomato Products.

Obtaining a Factory Background. When an analyst is assigned microscopic work on the detection of rotten tomatoes, he should first study rotten tomatoes from both a macroscopic and microscopic standpoint. One of the best ways to begin this work is to visit a tomato canning plant while it is in operation. The beginner visiting a large plant for the first time may be confused by the general plant conditions, usually markedly different from those encountered in the home. If the raw stock is poor, the canner is seriously handicapped in putting out a clean product. Solid-type rots showing discoloration may form in the field due to various diseased conditions. Excessive rains are especially damaging to the fruit at harvest time, since they may cause the fruit to crack open and thus allow the entrance of micro-organisms into the tomato tissues. Tomatoes may be cracked in handling and if not used promptly will rot. Long holding of the tomatoes usually results in the development of soft rots. A badly spoiled lot of fruit containing many affected with soft rot often resembles a mass of garbage rather than raw stock suitable for the preparation of a clean product. The beginner should study all of the rots directly from the field container. Every step in handling from that stage on serves to make the fruit "look" better, but makes the job of detecting its true condition more difficult. Solid rots show sufficient discoloration to enable the worker to detect the condition regardless of the amount of washing and handling. Soft rots on the other hand such as the oospora rot and the rhizopus or mucor rot may become "washed" to such an extent that it is difficult to detect the original conditions in the handled stock.

Causes of Rotting. Rotten tomatoes arise usually from the growth of molds which have gained entrance into the tomato through some defect in the skin. Field cracking following rains is the most common of such defects. So-called "cat-faced tomatoes" are tomatoes with brittle scar tissue radiating in varying ways from the blossom scar. Molds may enter more readily through the brittle rough coating than through the normally smooth peel of the tomato. In the same way when tomatoes have cracked open because of unbalanced moisture conditions, scar tissue may develop over the crack. While such a growth may develop in underripe fruit and tend to retard rotting, it is not safe

to assume that no rotting has occurred. It is therefore necessary to check tomatoes with light brown to brown scar tissue by cutting with a knife in order to determine whether rotting has begun beneath the scar. Frosted tomatoes, sun-scalded tomatoes, and tomatoes with blossom end rot are at the start free from mold. However, since the tissue has been killed in parts of the tomato, the dead tissue is more susceptible to attack by rot-forming molds. Tomatoes resting upon the soil often begin to rot at the site of contact. The point to bear in mind in studying the defects discussed above is that any one of a number of different molds or a combination of molds may cause the rotting once it has gained entrance into the tomato. In contrast to the above rots, there are a few organisms which cause rotting without the aid of obvious breaks in the skin. One of the common rots of this type is known as Anthracnose.

Appearance of Typical Rotten Conditions. Rot can usually be distinguished from sound tissue by its color. Most striking of the rots are those which are deep black in color. Such rots are usually to be found at or around stem-end cracks. From this marked discoloration rots vary in color to a light tan discoloration.

In the *Rhizopus* and *Mucor* type of rot, characterized by a marked softening of the tissue, the color may not differ greatly from the normal, although in general it will be a dead pink shade which can be distinguished from sound tissue by close observation. The importance of this observation is that a soft rot due to the *Rhizopus* or *Mucor* type of mold will be an obviously unfit tomato but after washing and handling its true condition may be so masked that it becomes difficult to distinguish without close scrutiny. The investigator should examine the raw stock at each plant in order to evaluate properly the washed and sorted stock. Another clue to the presence of the soft rhizopus and mucor rot in prepared stock is the fact that the affected tissue holds together in a soft flabby mass when it is moved with the knife blade, while normal tissue will usually fracture in a characteristic manner.

The *Oospora* type of rot will be readily noted by the whiteness of the mold as it occurs on the cracked surfaces. This mold may be accompanied by other micro-organisms. This type of spoilage may also be difficult to distinguish after it has gone through a washer. The odor of rotting, however, is often an index of the true condition of the fruit and all questionable areas should be subjected to the odor test. Another clue which should not be overlooked is the condition of the tomato peel along the edges of the exposed area. In the case of a freshly cracked tomato, the flesh just beneath the skin will usually be present, whereas in rot progressing in a cracked tomato, the tissue beneath the skin will have been washed away and the skin left in a wrinkled or even yellowish condition.

The essential point in the above discussion is that solid rots, usually discolored, will come through most handling unchanged, but that the soft rots may be so altered by the handling that close observation is required for their detection. Bear in mind that the canner usually depends upon washing to remove the soft rots. Very few canners sort the tomatoes in the dry condition when their true character can be determined. As a matter of fact, washing does remove most of the telltale evidence of the unsavory condition of the fruit but does not necessarily make the stock clean.

Common Molds Encountered in Rotting Tomatoes.

The following molds are the ones most likely to be encountered in rotten tomatoes:

a. *Alternaria* produces a black rot, growing into such defects as cracked or sunburned areas. The mycelium is septate and the spores are typical Indian-club-shaped. This fungus also causes a rot known as nailhead spot.

b. *Colletotrichum* produces at first shallow round spots on the surface. The disease is known as anthracnose. As the infection increases the discolored surface area becomes larger and the rot extends more deeply into the tomato. The anthracnose spot is light brown to tan and frequently the area is divided into concentric rings due to a difference in shade between the growth rings. The mycelium is fine, often markedly septate, and the spores are small and elliptical in shape. The mold growth occurs beneath the peel and is not aerial at any time.

c. *Fusarium* grows in cracks or injured spots and usually produces a soft rot with an aerial mycelium arising from the rot. Canoe or lunar-shaped spores are formed in the aerial mycelium.

d. *Mucor* produces soft rots by killing the tomato tissue. Tomatoes affected by mucors soon shrink and become a soft, pulpy mass. A basket of such tomatoes soon becomes totally unfit for use. The mold grows into the tissue and also grows aerially in a loose mass of mycelium. Sporangia are produced aerially and release millions of spores. When fruiting the *Mucors* are grayish-black in color. The mycelium is coarse, heavily granular, and non-septate.

e. *Rhizopus* is similar in appearance and effect to *Mucor* mold and can be distinguished only by the method of sporangiophore formation, as pointed out in the general discussion of the fungi.

f. *Oospora* grows on cracked, mashed tomatoes forming a white layer. The mycelium occurs in short pieces and readily breaks up into many blunt-ended spores. This mold as it occurs on tomatoes superficially resembles yeast because of the mass of spores produced. On machinery the *Oospora* mold differs in appearance as a result of the tapering of the branches and an apparent decrease in amount of sporulation.

g. Phoma rot attacks both the green and ripe fruits. On the green fruit the Phoma rot forms a small, slightly sunken area. As these areas enlarge they develop brown or black borders with slightly lighter colored centers. On ripe fruits the earlier stages show water-soaked concave areas. As the growth of the fungus increases the edge remains slightly sunken and discolored, while the center becomes charry black, firm and leathery. Black pimple-like fruiting bodies are scattered over this center portion.

Infection of fruits takes place usually through cracks or breaks in the skin, mainly around the stem end. The fungus may during the winter in the soil form a source of infection for the new crop of tomatoes. Fruit picked from infected fields, even though appearing sound when picked, may develop rot spots in 4–5 days. Spotted fruit may be used for food if used immediately and the diseased areas cut out. If kept for any period of time, the spots enlarge rapidly and the whole fruit becomes infected.

h. *Rhizoctonia solani* (Soil rot). Tomato fruits may become infected with a soil rot disease when they are produced under heavy vines and

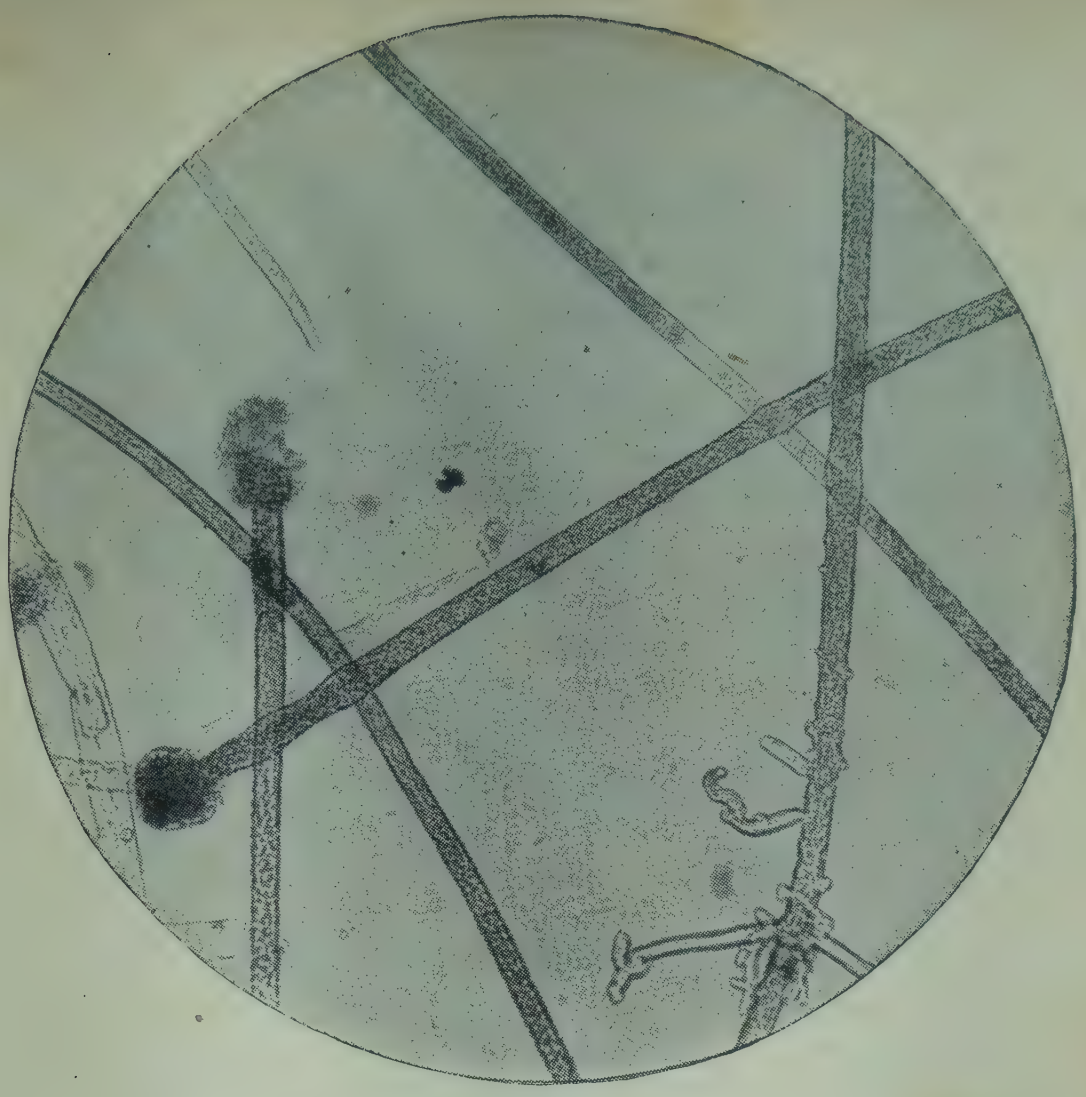

FIGURE 103.—*Rhizopus* sp. showing granulation.

FIGURE 104.—*Oospora* sp. from machinery.

in contact with wet soil. The fungus produces small, circular, brown spots on the lower surface of the fruits. These spots enlarge and show alternating areas of light and dark zones with radiating cracks. The fungus penetrates the tissues beneath the surface. It spreads rapidly through the tissues. In general, there is no appearance of mycelium on the surface of the infected fruits in the early stages but in the more advanced stages of growth it may be present if the tomatoes are moist or if they have been wrapped.

i. *Phytophthora terrestria* (Buckeye rot) is a type of rot affecting tomatoes in all stages of their development, and usually invading those that are in contact with the soil. The fungus is able to penetrate the epidermis of the fruit and is not dependent on mechanical injuries. On fruits it forms a brownish-colored area. As this area increases in size it produces a series of irregular brownish and light-colored concentric bands. The fungus spreads rapidly and causes breakdown of the tomato tissues in a short time. It has been observed that fruits may become completely rotted with no definite markings upon them except a solid, dull-brown color. Like the soil rot fungus, it develops faster in wet soils.

Mold Growth on Equipment. Mold, yeast, and bacteria have been found on factory machinery in the form of slime. The most commonly encountered mold in machinery slime is the *Oospora* type. It is apt to occur where the equipment has been constructed so as to make it difficult to reach all surfaces readily for cleaning. Complicated conveyors and inspection belts are conducive to this type of contamination. Metal chutes leading from inspection tables to conveyor belts, header vats used for holding raw juice, the insides of peeling pans, screw conveyors, composition belts, and stainless steel inspection tables have been found with a heavy mold growth. The investigator should look at all surfaces exposed to raw tomato juice or tomatoes. Surfaces upon which juice may splash are more apt to show mold growth than surfaces exposed to wear from the tomato load.

Often the odor from the slime is sufficient to indicate the presence of the contamination. When there is a splashing from the tomatoes, the mold may be hidden by the tomato debris but usually it is white in appearance and adheres tenaciously to the surfaces. A simple test for presence of slime is to scrape the questionable surface. The slime may be scraped up into a whitish compact mass with a knife blade or scalpel. The analyst should of course examine this material microscopically for mold to substantiate the scrape test.

Methods Used for Detection of Rot in the Finished Product. With a background of factory conditions in mind the analyst will be inclined to regard the techniques of the methods used for the detection of rot with interest. He is then not merely making a routine laboratory examination, but is going through a series of specific tests whereby he will be able to picture in his mind the true condition of the product.

Mold Count. The official method for the microanalysis of tomato products for mold is described in the Official and Tentative Methods of Analysis of the A.O.A.C., 5th Ed., pp. 522–524. This method is also given in the Manual of Microanalytical Methods as M13B.

The Rot Fragment Count. This method is described in the Manual of Microanalytical Methods.

Laboratory Training in the Examination of Tomato Products. The new analyst should make a careful study of the sound tomato

fruit and learn from experience with authentic material the appearances of the various anatomical and histological features of the tomato. The worker should pay particular attention to those structures which might be mistaken for mold such as the edges of the various types of cells, hairs on the seeds, and fibrovascular bundles. Samples of decomposed tomatoes should also be studied microscopically to determine in what respect they differ from sound tomatoes. The importance of this part of the training cannot be overemphasized as it is fundamental to the analyst's interpretation of his results on the finished product. He should remove material from various portions of the rotten areas, noting that while the evidence of rotting may precede the infection by the mold, the mold does not occur without leaving some physical evidence of its presence.

In the examination of the product there are some points which may be helpful in the identification of mold. These are described below:

The Identification of Mold Hyphae. Hyphae as a general rule occur in comminuted foods unaccompanied by fruiting bodies. Since the identification of specific molds is based for the most part on the type of fruiting body produced, it is generally impossible to determine from the hyphae the kind of mold present. However, it is important that the analyst be able to differentiate between mold hyphae and the normal elements found in the product. As mentioned above, if the counting is on a comminuted fruit or vegetable product, he should assure himself, by microscopical examination, of the basic fact that the rot itself consists of the fruit tissue and mold. The analyst should examine the mold hyphae and determine those features whereby he can identify the hyphal fragments and clumps when the fruit is pulped. Mold hyphae in all cases are tubular, although they may appear to be flat under the microscope. In most instances the diameters of the tubes are uniform, and hence the cell walls appear under the microscope as parallel lines. Two conspicuous exceptions are the molds of the *Mucor* type and the *Oospora,* where the hyphae are often tapering.

Granulation. Growing molds have living protoplasm within the tubular structure. In the growing portions of the mold the protoplasm may fill the entire space or it may surround vacuoles of cell sap. In either case, the protoplasm usually presents a granular or stippled appearance. This character may persist after the mold is killed in processing, or the protoplasm may coagulate into nongranular hyaline masses or plugs within the mold hyphae, leaving a considerable length of the tube free from protoplasmic material and thus empty in appearance.

Granulation is prominent in the *Mucor* and *Rhizopus* molds and may be indistinct in others. There may be only traces of protoplasmic material in many filaments of the molds frequently found in butter.

Septation. Most molds encountered in foods contain cross walls. These may be thought of as walls separating the tubes into sections. Such molds are spoken of as being septate. The presence of cross walls may serve to positively identify otherwise doubtful filaments. However, cross walls are generally absent in *Mucor* mold.

Branching. Most molds show an abundance of branching. Branching is frequently an aid in the positive identification of mold, although often fragments of mold are too short to show branching.

XIV. REGULATORY CONTROL OF FOODS AND DRUGS

In the foregoing text an attempt has been made to give to the inspector and analyst some fundamental information regarding the work which they have chosen for a career. More than ever before, the Food, Drug, and Cosmetic Act of 1938 gives the Food and Drug Administration powers for controlling sanitation in the food, drug, and cosmetic industries. Basically the Administration in carrying out its prescribed duties under the law has as its main objective protection of the consumer and from a sanitary standpoint, which includes not only the principles and objectives outlined in the first chapter of the text but also the macroscopic and microscopic filth which is discussed in other chapters, attention is focused on sections 402(a) (3), 402(a) (4) and 404(a), (b), and (c) of the law which deals with "pollution," "filth," and "disease" in foods, and the corresponding sections under chapters V and VI dealing with adulterated drugs (section 501) and adulterated cosmetics (section 601). Under section 402(a)(3), a food shall be deemed to be adulterated "if it consists in whole or in part of any filthy, putrid, or decomposed substance, or if it is otherwise unfit for food" and under 402(a)(4) "if it has been prepared, packed, or held under insanitary conditions whereby it may have become contaminated with filth, or whereby it may have been rendered injurious to health." These sections of the law can be used to advantage to bring about improvement in those industries where, through improper handling, filth and pollution and possibly dangerous pathogenic organisms may have been allowed access to the products involved. Furthermore, an extremely powerful regulation for the protection of the health of the consumer rests in section 404 which grants authority to the Federal Security Administrator, to establish an Emergency Permit Control over an industry when, after an investigation, the Administrator finds "that the distribution in interstate commerce of any class of food may, by reason of contamination with micro-organisms during the manufacture, processing, or packing thereof in any locality, be injurious to health***."

These sections of the Act which deal with the type of adulteration about which this text has been written do not call for nor in fact allow any so-called "tolerances" for filth and decomposition. The Circuit Court of Appeals (9th Circuit) in the case of A. O. Anderson & Co. versus United States, in discussing the fact that the Government witnesses had testified that they had heretofore allowed the food product in question, containing a small percentage of filthy, decomposed, or putrid matter, to pass in interstate commerce unchallenged, said "There is no room for controversy over percentages under the statute itself, for it excludes all." The inspector and the analyst should keep before them at all times the axiom that in the manufacture of a food or drug product it is the sole responsibility of the producer to see to it that the product that he is preparing for the consumer is as free from contamination as it is possible to attain. Obviously, in a commercial operation certain conditions may result in chance or accidental contaminations. However, these conditions, which are well recognized by the Administration and should be recognized by any fair appraisal, do not constitute a basis for setting up, for the benefit of the industries involved,

"tolerances" for filth for the careless producer to shoot at. Fortunately, in the food and drug industries as a whole there are many producers who are well aware of their responsibilities and are not interested in the setting up of tolerances for filth and adulteration, but are only interested in getting out what they consider to be the very best product they are capable of producing. As a matter of fact, the majority of food and drug manufacturers in this country are producing foods and drugs in as scrupulously clean and desirable a manner as the consumer would expect. The inspector making an inspection must keep in mind what is considered to be good commercial practice in judging the operations in a given plant. His judgment should always be based on a wealth of experience in the food or drug industry of which the plant is a representative. Similarly, the analyst in his examination of a food or drug product collected by the inspector must give consideration, in drawing his conclusions, to the extent of the contamination he has been able to demonstrate in similar food and drug products. Even though the analyst is not responsible for the action that may be taken by the Administration, he is responsible as an expert for the interpretation of his findings.

To those interested in the field of microanalysis of food and drugs it will become obvious from a perusal of the foregoing text that from a regulatory standpoint the field is extremely broad. The text cannot cover thoroughly and in detail all of the various phases of microanalysis that can be used to assure the consumer of a clean and unadulterated food or drug product. However, it is believed that the text will be of value to the inspector in his sanitary inspection of plants, to the analyst in his daily examination of samples, and to the regulatory official whose prime interest is in making it possible for the consumer to obtain food and drug products that are free from adulteration.

XV. APPENDIX

M2B

Filth in Flour

The basic method commonly has been known as the "saturated-salt gasoline-flotation" method and was designed to recover insects and insect fragments from white wheat flour. In this writing there is included an optional procedure for the sedimentation of heavy filth such as rodent pellets and stones. The two procedures may be run in conjunction with each other or each may be carried out separately. A rapid method is provided for separating out whole insects and other gross evidence of contamination more rapidly when the samples are so severely contaminated that a less detailed examination will suffice. In either case, record the method used.

(1) SATURATED-SALT GASOLINE.

Weigh 50 gm. of flour in a 250 ml. beaker and transfer to a 2-liter Wildman trap flask containing 200 ml. of filtered saturated salt (NaCl) solution. The neck of the flask must be dry to prevent the flour from sticking to it. Break up the lumps by stirring with the stopper until a homogeneous mixture is obtained. Add 35 ml. of gasoline and mix in with a gentle rotary motion. Too vigorous stirring results in the formation of a dense emulsion which causes the final paper to contain such an amount of flour that the filth particles are mashed. Vigorous stirring at this point is unnecessary since most of the elements of filth will float in saturated salt solution.

Add salt solution slowly by pouring it down the side of the flask and stir gently until the flask is filled. Trap off the gasoline layer to another trap flask approximately ⅓ full of water or saturated salt solution. For this rewashing of the sample use either a 1-liter or 2-liter trap flask. The trapped off material may be transferred to a beaker, the trap flask rinsed, and the material placed back in the same flask. Trap off from the second flask, filter, and examine.

M4B

Filth in Cheese Products

Cheese may be contaminated with insect and/or rodent filth during the handling of the milk, during the manufacture of the cheese, or during curing. Where it is essential that an accurate picture of the amounts of such filth be obtained a procedure given under "Quantitative procedures" should be used. It will be noted that a number of reagents are listed. In general the choice of reagent will depend upon the type of cheese and the past experience of the analyst. Where a number of samples are to be run, preliminary tests by one of the methods listed under "Qualitative procedures" may be found to be advantageous and more useful as a sorting procedure. Where the main purpose is to obtain sediment comparison one of the qualitative procedures may be used. In all cases use a sample of 225 gm.

(1) QUANTITATIVE PROCEDURE.

Preparation of sample. Cut cheese into cubes 25 mm. across or smaller and add to the reagent selected in an appropriate-size beaker. Provide means of heating or maintaining proper temperature and means of stirring the mixture similar to that specified in A.O.A.C. Methods of Analysis, 5th ed., Chapter XXII, paragraph 97.

(a) Sodium citrate.

Use 400 ml. of 15% sodium citrate in 1,500-ml. beaker and heat to 65° C. Add cheese and stir. Maintain temperature close to 60° C. but not above 62° C. After stirring 5 to 15 minutes, add 200 ml. H_2O and 200 ml. sodium citrate solution both heated to 60° C. Continue stirring 5 to 15 minutes longer and filter through paper, keeping a stream of hot water on the paper to facilitate filtration.

M4C

Filth in Processed Milk

These methods include dried milks, evaporated milk and condensed milk. All types may be contaminated with filth from the barn, utensils, and while enroute to the processing plants. The filth encountered may include insects, rodent hairs, and nondescript dirt and manure particles.

(1) (b) Sediment pads.

To 300 ml. of cold water in a Waring Blendor gradually add 100 gm. of the milk powder. Avoid prolonged mixing because of excessive foam formation. The total mixing time need not exceed 15–20 seconds. Transfer the mixture to a liter beaker, rinsing the blendor with about 150 ml. of water. Stir into the milk mixture 100 ml. of filtered 40% sodium citrate solution. Heat to 60° C. while stirring and maintain at this temperature with frequent stirring for 10–15 minutes or until the milk powder is dissolved and the solution appears translucent. Filter the hot solution on a milk sediment pad with a cloth covering. Any foam on the solution will pass through the pad without difficulty. Use a little hot water from a wash bottle to assist in transferring foam and overcoming any tendency of the filtration to slow down.

M4E

Mold and Filth in Butter

(1) MOLD

A high mold count in butter indicates the use of decomposed cream in its preparation. Make a careful examination of the surface of the sample and note any visible mold growth. To remove the possibility of contamination by surface mold, scrape off and discard ⅛″ of surface. Weigh out 1 gm. of butter obtained from exposed surface in a tared ¼-teaspoon measure and place spoon in a 50-ml. beaker. Add 7 gm. of hot (50°–60° C.) gum solution prepared by making either a .75% solution of carob bean gum or a 3% solution of pectin or other gum

solution of similar viscosity. To prepare solution, make a thin paste-like mixture of the required amount of the dry powder in alcohol, add cold water, mix, and heat gradually. Allow to boil until gum is dissolved. Adjust volume, if necessary, after cooling. Preserve with 2% formaldehyde solution U.S.P. In the case of carob bean gum or other gum allow cellular elements in the mixture to settle out and use clear supernatant fluid. Stir until mixture is uniform and fat globules are 0.1–0.2 mm. in diameter. Mount a portion of the mixture on the mold-counting slide and estimate mold as directed under A.O.A.C. Methods of Analysis, 5th ed., Chapter XXXV, paragraphs 30, 31. Consider fields positive when a single filament or combined length of the two longest filaments exceeds $\frac{1}{6}$ of diameter of field.

(1) (a) Alternate procedure (Staining).

Add 1 or 2 drops of 5% crystal violet solution to the gum-butter mixture after butter is melted. Mix preparation thoroughly and prepare slide as directed above.

M7A

Filth in Spices

(2) ROT IN POWDERED CAPSICUM (BASED ON MOLD COUNT).

Occasionally an excessive number of moldy pods are used in preparing ground capsicum products such as chili powder, cayenne pepper, red pepper, paprika, etc. This adulteration may be determined by the following procedure:

PROCEDURE REVISED:

Weigh out 10 gm. of the thoroughly mixed sample of ground capsicum and transfer to a Waring Blendor. Add 200 ml. of a 1% NaOH solution in three or four successive portions, stirring the mixture upon each addition, and finally wash down with the final portion any material that may stick to the walls of the blendor. Agitate the mixture in the blendor for 1 minute. Rub down into the mixture any material sticking to the walls of blendor with a rubber policeman and repeat the blending for 2 minutes longer. Add 2 or 3 drops of caprylic alcohol to break the resulting foam. Mix 100 gm. of this mixture with 50 gm. of a 4% pectin solution[4] and count with the Howard mold counting chamber as for tomato products.

Occasionally a blended mixture will contain particles of seed tissue which make it difficult to obtain "Newton rings" in preparing the slide for mold counting. A clamp for holding the cover slip in place has been devised which removes this difficulty. This consists of a metal plate with a circular opening, 2.5 cm. in diameter, in the center of the plate. Two clips attached to the anterior edge of the plate fasten the cover slip in position when the slide is placed on the plate.

[4] Weigh 4 gm. of powdered pectin and add sufficient alcohol to make a thin paste. Add, with stirring, 100 ml. of cold water and heat gradually. Boil until the paste is dissolved and adjust the volume to 100 ml. after cooling. Add 2 ml. of formaldehyde solution U.S.P.

M8B

Rot in Blackberries and Raspberries

Canned and frozen blackberries may contain some rotten fruit which may be detected by a careful macroscopic examination of the berries under water after they have been washed. Frequently canned blackberries will disintegrate to some extent and the detection of rot in this material presents a special problem.

PROCEDURE.

Drain the contents of a No. 2 can or its equivalent on a #20 sieve (5″ diameter). Immerse the berries in water in a large white pan. Pour off most of the water through a #20 sieve and add more water. Repeat the washing if the water is not fairly clear. Examine the berries under water and remove all questionable berries to a black pan containing distilled or deaerated water. Reexamine the suspected berries and note particularly the outline of the berries as they are turned over under a strong beam of light. Class as rotten, berries and fragments which have at least 3 drupelets containing either external (aerial) or internal mold, or both. Separate into two classes—those with external mold, and those moldy but without external mold. Confirm all questionable rot spots by examining a fragment of the tissue for mold under the compound microscope. Classify the tissue as rotten only when a substantial number of mold filaments is present.

Drain separately the good, and the two separations of rotten berries and fragments for 2 minutes on a #20 sieve (8″ diameter). Weigh each rotten portion separately and add these to the good berries.

If the sample contains a large amount of disintegrated berry material, pick out the whole berries and large fragments. Remove the disintegrated material to a #20 sieve and allow it to drain for 2 minutes and then weigh. Take an aliquot of approximately one-fourth and separate the rotten from the good material. Calculate the total weight of rot in all the disintegrated material and add this to the weight of whole berries showing internal mold. Mix all the portions together, drain, and determine the total drained weight of the sample. Calculate and record the percentages of external and internal mold. Pulp the berries through a cyclone with openings ca. 0.023″ in diameter, or through a #30 sieve using a stiff brush. Mix the pulp thoroughly, weigh out 50 gm., and dilute with an equal weight of pectin solution. Make a mold count on this mixture.

M8C

Rot in Strawberries

Strawberries are most commonly received for examination in the frozen condition. Some of the berries may contain rotten areas. These may be detected by a careful macroscopic examination of the berries under water after they have been washed with water. The berries

should be thawed either at room temperature or in an electric refrigerator at ca. 40° F. Do not immerse the sample in hot water, since this makes the berries soft and mushy.

PROCEDURE.

Drain the entire sample, if 1 quart or less, on a #20 sieve (5″ diameter). Immerse the berries in water in a large white pan. Pour off most of the water through a #20 sieve, catching and returning any strawberry tissue, and add more water. Repeat the washing if the water is not fairly clear. Examine the berries under water and remove all questionable berries to another pan containing distilled or deaerated water. Reexamine the suspected berries. Class a berry or fragment as rotten if it has a rot area at least 6 mm. in diameter. Confirm all questionable rot spots by examining a fragment of the berry tissue for mold under the compound microscope. Classify the tissue as rotten only when a substantial number of mold filaments is present. Also separate and count berries and/or fragments with rot areas 12 mm. or more in diameter. Count all rotten berry fragments in the above classes and enter the count in the table. Drain separately the good and rotten separations for 2 minutes on a #20 sieve (8″ diameter). Weigh the rotten separation, add this to the good, and determine the total drained weight of the sample. Pulp the berries through a cyclone with openings ca. 0.023″ in diameter, or through a #30 sieve using a stiff brush. Mix the pulp thoroughly, weigh out 50 gm. and dilute with an equal weight of pectin solution. Make a mold count on this mixture.

Insect Fragments and Other Light Filth in Fig Paste

PROCEDURE.

Place 100 gm. of the sample in a beaker with ca. 300 ml. of water. Boil and stir until the paste is thoroughly softened and mixed with water. Transfer to a 2-liter Wildman trap flask, add 20 ml. of castor oil, and stir thoroughly. Add sufficient warm tap water (50°C.) to fill the flask. Allow to stand for at least half an hour with occasional stirring. Trap off the oil layer. Add a little water to the flask, stir, and trap off again after 10 minutes. Filter the trapped off portions through a rapid paper and examine with a Greenough microscope at 20 to 30 diameters.

M13D

Rot Fragments in Tomato Products

Weigh 10 gm. of juice (5 gm. of puree or catsup or 2 gm. of paste) into a 50 ml. tared beaker and rinse with 100 ml. tap water into a 400 ml. beaker. Add ca. 2 ml. saturated aqueous gentian violet solution, stir, and allow to stain for 3 minutes. Add 200 ml. of water, stir, and pour through a #60 sieve ca. 7.5 cm. in diameter. Keep sieve in horizontal position set in the circular opening of holder[5] over a 1-liter

[5] Make a suitable holder for sieve by cutting a circular opening in a rectangular piece of tin slightly larger in diameter than the outer diameter of the lower part of the sieve. Bend corners of tin down to fit a 1000-ml. beaker and trim to convenient size.

beaker. In pouring, distribute material over entire surface of sieve to insure rapid drainage, using a glass rod held against the lip of the pouring beaker with the lower end of the rod ca. 2 cm. from the screen. (If sample weight given above does not drain rapidly, reduce size of sample and take this into consideration in computing number of fragments.) Rinse beaker with two 100-ml. portions of tap water pouring each portion over the strained tomato debris on the sieve using the glass rod as before. Tilt sieve in holder to about a 30° angle and wash debris to the lower part with water. This usually requires ca. 100 ml. Allow debris to drain and transfer as much as possible to the bottom of a 50 ml. centrifuge tube with a thick, metal, square-ended spatula ca. 6 mm. wide. Wash remaining debris again to lowermost part of screen and add to centrifuge tube as before. This procedure may be repeated until most of the debris has been transferred to the tube. Transfer the final remaining debris by means of a pipette, ca. 20 cm. in length, 6 mm. inside bore, fitted with a rubber bulb. To do this wash debris down with water from pipette and immediately take up debris in wash water before it has run through screen. Usually 300 ml. of water will be ample for the complete transference of the tomato debris from the sieve to the centrifuge tube. When completely transferred, the volume of water and debris should be made up with water to 10 ml. Add sufficient neutralized 3% algin solution to make volume up to 20 ml. The clear gum solution prepared from carob bean gum may also be used for this purpose [M4E (1)]. Mix stained suspension with spatula and measure out two separate 0.5-ml. amounts to each of two counting plates and cover with special cover slip. (The pipette is prepared by cutting off a 1-ml. pipette squarely at the 1-ml. mark.) In pipetting, draw the material slightly above the 0.5-ml. mark and then allow it to drop slowly back to the proper volume. Wipe off the pipette and then allow the material to flow slowly onto the slide spreading uniformly in the center of the slide covering an area roughly 6 cm. x 2 cm. Touch lower end of pipette several times to slide to insure removal of material. Blow out last drop if necessary. Examine each slide with Greenough type microscope using a magnification of ca. 40–45 diameters with transmitted light. The microscope should be fitted with a 4X bi-objective and 10X oculars or a similar combination. For the light source use a substage box-type light, with a 15-watt bulb, and blue or daylight ground-glass filter. Place light so that center of glass filter is directly below objective and ca. 2 cm. below glass stage of microscope. Count and record the number of rot fragments on each of the two slides, add, and multiply the sum by 2 (10-gm. sample) to obtain number of rot fragments per gram of product. Where 5 gm. of material is used multiply total of the two plates by 4 and by 10 where a 2-gm. sample is used. (If it is found necessary to use a different size sample, calculate appropriate factor.)

Count as rot all fragments showing abundance of mold with tissue either apparent or present in slight amounts.

Many of the fragments observed will be heavily stained masses of mold filaments which are obviously rot. Because of the thickness of the